



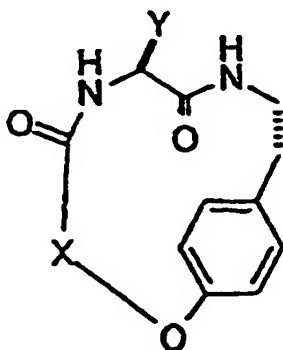
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: C07D 273/02, 413/06, 413/14, 513/08, 487/08, C07K 5/023, 5/078, 5/097, 7/06, 5/06	A1	(11) International Publication Number: WO 96/16950 (43) International Publication Date: 6 June 1996 (06.06.96)
(21) International Application Number: PCT/AU95/00817 (22) International Filing Date: 4 December 1995 (04.12.95) (30) Priority Data: PM 9825 2 December 1994 (02.12.94) AU (71) Applicant (for all designated States except US): THE UNIVERSITY OF QUEENSLAND [AU/AU]; St. Lucia, QLD 4072 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only): ABBENANTE, John [AU/AU]; 38 Queenscroft Street, Chelmer, QLD 4068 (AU). BERGMAN, Doug [AU/AU]; 67 Whitmore Street, Taringa, QLD 4068 (AU). BRINKWORTH, Ross [AU/AU]; 8/16 Dovercourt Road, Toowong, QLD 4066 (AU). DANCER, Robert [AU/GB]; Dept. of Chemistry, Lensfield Road, Cambridge CB2 1EW (GB). GARNHAM, Bronwyn [AU/AU]; 11 Foraker Drive, Eagle Heights, QLD 4271 (AU). HUNT, Peter [GB/GB]; 26 The Lanes, Over Cambridgeshire CB4 5NG (GB). FAIRLIE, David [AU/AU]; 73 Trevallyan Drive, Springwood, QLD 4127 (AU). MARCH, Darren [AU/AU]; 81 Ninth Street, St. Lucia, QLD 4067 (AU). MARTIN, Jennifer [AU/AU]; 105 Payne Street, Indooroopilly, QLD		4068 (AU). REID, Robert [AU/AU]; 8 Oak Avenue, Browns Plains, QLD 4118 (AU). (74) Agent: KELLY, Robin, Thomas; Fisher & Kelly, Level One, 349 Coronation Drive, P.O. Box 1477, Milton, QLD 4064 (AU). (81) Designated States: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG). Published With international search report.

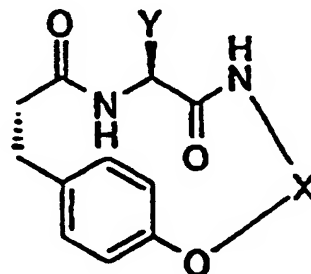
(54) Title: HIV PROTEASE INHIBITORS**(57) Abstract**

A HIV-1 protease inhibitor which includes an N-terminal cycle (A) or a C-terminal cycle (B) or both cycles (A) and (B) wherein Y is selected from side chains of Asn or Ile or Val or Glu and alkyl of 1-6 carbon atoms inclusive of linear or branched chains as well as cycloalkyl; and X is selected from (CH₂)_n where n = 3-6, -CH(OH)-CH(OH)-CH₂-, CH(CO₂H)-CH₂-CH₂-,

CH₂CONHCHR where R = D or L amino acids and alkyl of 1-6 carbon atoms inclusive of linear or branched chains.



(A)



(B)

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

TITLE

"HIV PROTEASE INHIBITORS"

FIELD OF THE INVENTION

THIS INVENTION relates to HIV protease inhibitors.

5

BACKGROUND OF THE INVENTION

In recent years, the worldwide research focus on the development of anti-HIV drugs has tested the viability of receptor-based drug design and has helped to focus on *de novo* drug design. The proteinase of the Human Immunodeficiency Virus, HIV-1 protease (Blundell *et al.*, 1990, TIBS 15 425-430; Huff, J., 1991, J. Med. Chem. 34 2305-2314; Darke, P.L. and Huff, J. R., 1994, Adv. Pharm. 25 399-454; Debouck, C., 1992, AIDS Res. & Hum. Retrovir 8 153-164), is a conspicuous example of a receptor for which drug design methodologies have been applied with some success. The rational design of clinically effective inhibitors for this enzyme has remained elusive.

The Human Immunodeficiency Virus (HIV) now infects over 15 million people worldwide, including some 20,000 individuals in Australia. Drugs aimed at treating HIV infection are being rationally developed to target key regulatory proteins that are essential for the replication of HIV. One of these proteins, HIV-1 protease (HIVPR), is an aspartic protease (James, M.N.G. & Sielecki, A.R., 1989, in Biological Macromolecules & Assemblies (Jurnak, F.A. & McPherson, A.M., eds), Wiley, N.Y., Vol. 3, p. 413; Fitzgerald, P.M. & Springer, J.P., 1991, Ann. Rev. Biophys. Chem. 20 299-320) that acts late in the viral replicative cycle by processing polypeptides (Pr160 and Pr50) transcribed from the *gag* and *pol* genes. The protease is essential for assembly and maturation of infectious virions but becomes inactivated by a single mutation (Asp25) in the active site, resulting in immature, non-infective virus particles (Kohl *et al.*, *supra*; Ash rn *et al.*, Proc. Natl. Acad. Sci. U.S.A. 87 7472-7476; Lambert *et al.*, 1992, Antimicrob. Agents. Chemother. 36 982-988). Since inhibitor binding

to HIVPR can also prevent infection of immune cells by HIV, the protease is a valid target for chemotherapeutic intervention (Kohl, N.E., 1988, Proc. Natl. Acad. Sci. USA 85 4686-4690; Ashorn *et al.*, supra; Lambert *et al.*, supra). Inhibitors of HIVPR can be expected to be possible treatments for HIV-infections. Indications are that resistance is more difficult to develop against HIVPR inhibitors (Roberts *et al.*, 1990, Science 248 358-361; Craig *et al.*, 1991, Antiviral Res. 16 295-305; Muirhead *et al.*, 1993, 9th Intl. Cong. AIDS (Berlin), Abs PO-B30-2199; Muirhead *et al.*, 1992, Br. J. Clin. Pharm. 34 170-171; Roberts *et al.*, 1992, Biochem. Soc. Trans. 20 513-516) than reverse transcriptase inhibitors (Tomasselli *et al.*, 1992, Chimicaoggi 6-27), but resistance is still proving to be a major problem.

HIVPR is a homo-dimer, consisting of two identically folded 99 amino acid subunits that form a hydrophobic active site cavity. The C_2 symmetry of the enzyme is a unique feature among aspartic proteinases. HIVPR is also characterised by two conformationally flexible flaps (one per subunit) which are able to close around the substrate. The three dimensional crystal structures of both recombinant and synthetic HIVPR have been reported for the enzyme as well as enzyme-inhibitor complexes (Tozser *et al.*, 1992, Biochemistry 31 4793-4800; Swain *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87 8805-8809). The major difference between these enzyme conformations is in the location of the flaps and some residues in the hinge region. The amino acids of HIVPR that line the substrate-binding groove, which is 24Å long by 6-8Å diameter, are symmetrically disposed around the catalytic residues located near the centre of the active site.

The first approaches to developing inhibitors of HIVPR involved a combination of *analogue-based* and *mechanism-based* drug design that focused on the amino acid sequence of substrates for HIVPR. These inhibitors were based on the observed preference for

pr teolysis of substrates with a scissile hydrophobic-hydrophobic or
ar matic-proline peptide bond (Griffiths, J.T., 1992, Biochemistry 31
5193-5200) and were both potent and selectiv for HIVPR. Aside
from optimising the fitting of amino acid side chains into the
5 corresponding binding pockets (Wlodawer, A. & Erickson, J.W.,
1993, Ann. Rev. Biochem. 62 543-585 and references therein) that
line the substrate- binding groove of HIVPR, inhibitor design must also
take into account hydrogen bonding and electrostatic interactions
which occur along the binding groove.

10 Roberts *et al.*, 1992, Biochem. Soc. Trans. 20 513-516;
Pharmaprojects AN 017782 9202, PJB Publications Ltd., Richmond,
Surrey, U.K.; Paessens *et al.*, 1993, 9th Intl. Cong. AIDS (Berlin) Abs
PO-A25-0591 & PO-A25-0611; Pharmaprojects AN 019149 9212 &
AN 020519 9310, PJB Publications Ltd., Richmond, Surrey, U.K.;
15 206th ACS (Chicago), 1993, MEDI 138; 32nd ICAAC (Anaheim)
1992, Abs 315 & 1501; Getman *et al.*, 1993, J. Med. Chem. 36
288-291; Alteri *et al.*, 1993, Antimicrob. Agents. Chemother. 37
2087-2092; Pharmaprojects AN 020519 9310, PJB Publications Ltd.,
Richmond, Surrey, Kim *et al.*, 1993, 9th Intl. Cong. AIDS (Berlin) Abs
20 PO-A25-0622; Vacca *et al.*, 1994, Proc. Natl. Acad. Sci. USA 91
4096-4100; Pharmaprojects AN 020180 9307, PJB Publications Ltd.,
Richmond, Surrey, U.K.; Jadhav *et al.*, 9th Int. Cong. AIDS (Berlin)
Abs PO-A25-585; Vacca *et al.*, 9th Int. Cong. AIDS (Berlin) Abs PO-
B26-2023; Young *et al.*, 1992, J. Med. Chem. 35 1702-1709; Cohen
25 *et al.*, 1990, J. Med. Chem. 33 883-894; Thiasrivongs *et al.*, August
1994, Proc. 10th Int. Conf. AIDS (Yokohama) Abs 322A;
Pharmaprojects AN 018824 9209, PJB Publications Ltd. Richmond,
Surrey, U.K.; Kiso *et al.*, 1993, 9th Intl. Cong. AIDS (Berlin) Abs PO-
A25-567; 32nd ICAAC (Anheim), 1992, Abs 317-318; Kageyama *et*
30 *al.*, 1993, Antimicrob. Agents Chemother 37 810-817; Mimoto *et al.*,
1992, Chem. Pharma. Bull 40 2251-2253; Scrip-world
Pharmaceutical News, 1992, 1633 p 26; The Blue Sheet, 1992 35 p

- 8; Pharmaprojects AN 014606 9108, PJB Publications Ltd. Richmond, Surrey, U.K.; Danner *et al.*, 1993, 9th Intl. Cong. AIDS (Berlin) Abs WS-B2606; Kempf *et al.*, 1991, Antimicrob. Agents Chemother 35 2209-2214; Kort *et al.*, 1993, Antimicrob. Agents Chemoter 37 115-119.26; Kempf *et al.*, 1992, J. Org. Chem. 57 5692-5700; Kempf *et al.*, 1993, J. Med. Chem. 36 320-330; Erickson *et al.*, 1990, Science 249 527-533; Pharmaprojects AN 018825 9209, PJB Publications Ltd. Richmond, Surrey, U.K.; 8th Intl. Conf. AIDS (Amsterdam), 1992, Abs ThA1507; Melnick *et al.*, 1994, Proc. 207th ACS National Meeting (San Diego) Abs MEDI-20; Reich, S.H. 1994, Proc. of New Advances in Peptidomimetics and Small Molecule Design (Philadelphia); Sheety *et al.*, August 1994, Proc. 10th Int. Conf. AIDS (Yokohama) Abs 321A; Lam *et al.*, 1994, Science 263 380-384; Grzesiek *et al.*, 1994, J. Am. Chem. Soc. 116 1581-2 and Otto *et al.*, August 1994; Proc. 10th Int. Conf. AIDS (Yokohama) Abs 320A describe some of the more established potent inhibitors of HIVPR *in vitro*. Reference may be made to Smith *et al.*, 1994, Biorganic & Medicinal Chemistry Letters 4 No. 18 2217-2222 which refers to HIV proteases comprising conformationally constrained peptide based hydroxyethylamines with 17 to 19 membered macrocyclic ring systems. These inhibitors all require the decahydroisoquinoline ring at the C terminus and the large size of the ring system does not permit these compounds to specifically mimic the substrate(s) of HIV protease.
- 25 All of the inhibitors in the abovementioned references are potent inhibitors of infection of cultured human cells *in vitro*, although their potency is usually 1-2 orders of magnitude lower in cells than against HIVPR *in vitro*. This problem is also discussed in Rich *et al.*, 1990, J. Med. Chem. 33 1285; Toth *et al.*, 1990, Int. J. Peptide Protein Res. 36 544-550; Brinkworth *et al.*, 1991, Biochem. Biophys. Res. Comm. 176 241-246 and Majer *et al.*, 1993, Arch. Biochem. Biophys. 304 1-8. Most of these compounds are being or have been

investigated by pharmaceutical companies *in vitro* and *in vivo* and as prospective anti-viral drugs.

In contrast to reverse transcriptase inhibitors referred to in Tomasselli *et al.*, supra, and Petteway *et al.*, 1991, TiPS 12 28-34; Clercq, E.D., 1987, TiPS 8 339-345 and Field, H. & Goldthorpe, S.E., 1989, TiPS 10 333-337, protease inhibitors are able to block HIV infection in chronically as well as acutely infected cells which is crucial for clinical efficacy (Roberts *et al.*, 1990, supra; Craig *et al.*, 1991, supra; Muirhead *et al.*, 1993, supra; Muirhead *et al.*, 1992, supra and Roberts *et al.*, 1992, supra, for example.

All of the substrate-based protease inhibitors described in the abovementioned references which are potent inhibitors of HIV infection of cultured human cells *in vitro* suffer as drugs from a combination of pharmacodynamic and pharmacokinetic problems. These problems are also discussed in Field, H. & Goldthorpe, S.E., 1989, TiPS 10 333-337; Kageyama *et al.*, 1992, Antimicrob. Agents Chemother. 36 926-933; Sanders et al., 1993, Drugs 45 637-653 and PALLAS pKalc and PrologP available from CompuDrug Chemistry Ltd., Hungary. These problems include:-

- (i) short serum half lives ($t_{1/2}$) and high susceptibility to hydrolysis by degradative enzymes present in the bloodstream, gut and cells;
- (ii) poor absorption, low water-solubility and oral bioavailability; and
- (iii) rapid liver clearance and biliary excretion.

To improve their stability and bioavailability, various synthetic modifications need to be made but if these become too sophisticated, the economic feasibility of drug production can be compromised.

Structural modifications, involving polar ionisable groups that lead to increased gastrointestinal absorption and plasma concentrations of a reverse transcriptase inhibitor (Rosenberg *et al.*, 1991, J. Med. Chem. 34 469-471), are also being made to improve the

pharmacological profile of protease inhibitors. This approach is discussed in Flentge *et al.*, 1994, Proc. 206th ACS National Meeting (San Diego), MEDI-35.

5 A major strategy to reduce pharmacological problems is to develop inhibitors which either structurally or functionally mimic bioactive peptides but have reduced or no peptide character. Few non-peptide inhibitors have been reported to date. Some are described in Debouck, C., 1992, *supra*; Brinkworth, R.I. & Fairlie, D.P., 1992, Biochem. Biophys. Res. Commun. 188 624-630;; Lam *et al.*, 10 1994, *supra*; Grzesiek *et al.*, 1994, *supra*; Otto *et al.*, *supra*; Tung *et al.*, August 1994, Proc. 10th Int. Conf. AIDS (Yokohama) Abs 426A; DesJarlais *et al.*, 1990, Proc. Nat. Acad. Sci USA 87 6644-664; Rutenber *et al.*, 1993, J. Bio. Chem. 268 15343-15346 and Saito *et al.*, 15 1994, J. Biol. Chem. 269 10691-10698. This approach is described in Vacca *et al.*, 1994, *supra*; Pharmaprojects AN 020180 *supra*; Jadhav *et al.*, 1993, *supra*; Vacca *et al.*, 9th Int. Cong. AIDS (Berlin) Abs PO-B26-2023; Young *et al.*, 1992, *supra*; Tucker *et al.*, 1992, *supra* and Bone *et al.*, *supra* which refers to decrease in inhibitor size, which might theoretically minimise biliary excretion 20 while at the same time increasing water solubility. However, such an approach has so far met with limited success.

However, from the foregoing, it will be appreciated that prior art HIV-1 protease inhibitors which are mostly peptides or peptide derived in character have suffered from a number of 25 disadvantages which include:-

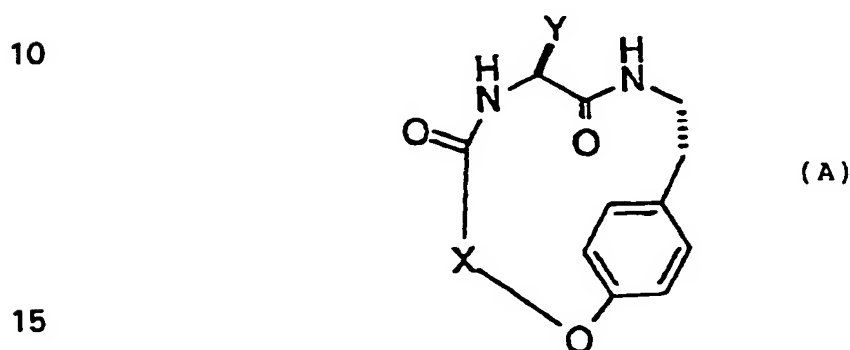
- (i) they are subject to proteolysis or hydrolysis by peptidases and therefore do not reach cells infected with HIV-1;
- (ii) they do not have a stable receptor-binding 30 configuration insofar as they are capable of many different conformations; or
- (iii) they are not anti-viral in nature, i.e. they do not

prevent viral replication.

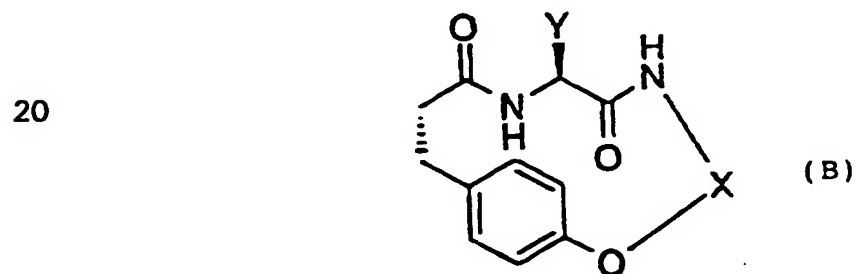
SUMMARY OF THE INVENTION

An object of the invention is to provide HIV-1 protease inhibitors that can structurally and functionally mimic the substrates of the enzyme HIV-1 protease and thus alleviate the aforementioned problems of the prior art.

Therefore, the invention provides HIV-1 protease inhibitors that include an N-terminal cycle



or a C-terminal cycle



25 or both cycles (A) and (B) wherein X and Y are as hereinafter defined.

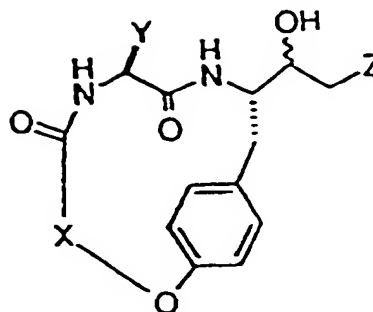
In regard to structures (A) and (B) above, it will be appreciated that such structures are the same with the exception that the two amide bonds are inverted.

Therefore, the invention includes within its scope, protease inhibitors having the following structures (i), (ii) and (iii) set out hereinbelow which include cycles (A) or (B) or both.

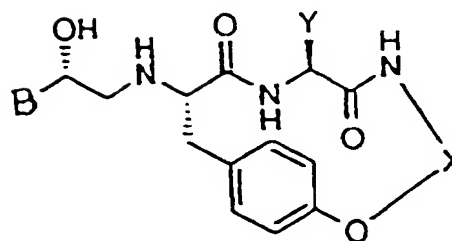
30

8

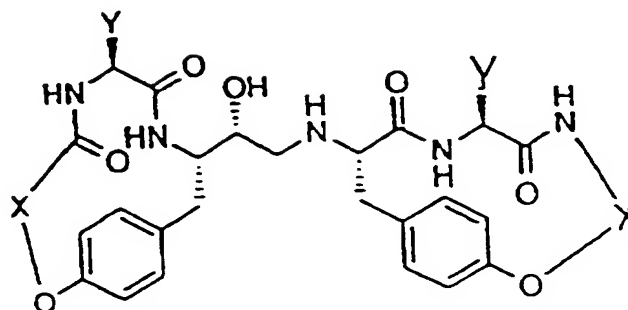
N-Terminus Cyclic Inhibitors (i)



C-Terminus Cyclic Inhibitors (ii)



Bicyclic Inhibitors (iii)



In the structures (i), (ii) and (iii) above,

X = $-(CH_2)_n-$ where $n = 3-6$ but is preferably 3, 4 or 5,
alkyl of 1-6 carbon atoms inclusive of linear and
branched chains as well as cycloalkyl
 $-CH(OH)-CH(OH)-CH_2-$,
 $-CH(CO_2H)-CH_2-CH_2-$, or
 $-CH_2CONHCHR-$ where R = D- or L- amino acids and
especially Lys, Arg, His, Tyr, Phe, Glu, Gln, Ile, Val or
Asp,

Y = side chains of Asn or Ile or Val or Glu; or

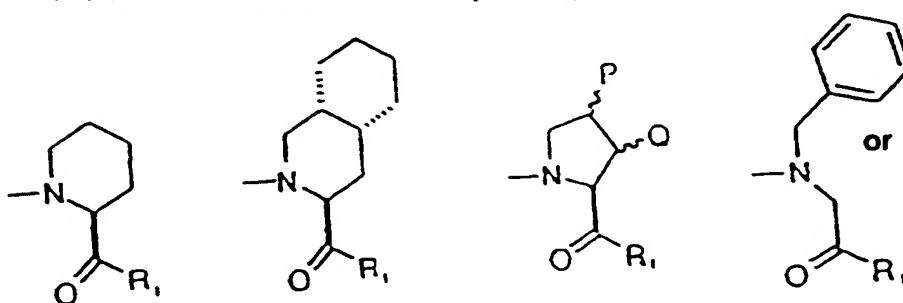
9

alkyl of 1-6 carbon atoms inclusive of linear and
branch d chains as well as cycloalkyl

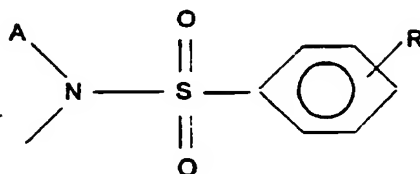
5

Z

=



10



15

wherein

P = H, alkyl, aryl, Oalkyl, Nalkyl

Q = H, alkyl, aryl, Oalkyl, Nalkyl

R₁ = NHtBu, OtBu, NHiBu, NHiPr, NHnBu, NHnPr, NHalkyl, Oalkyl; or

20

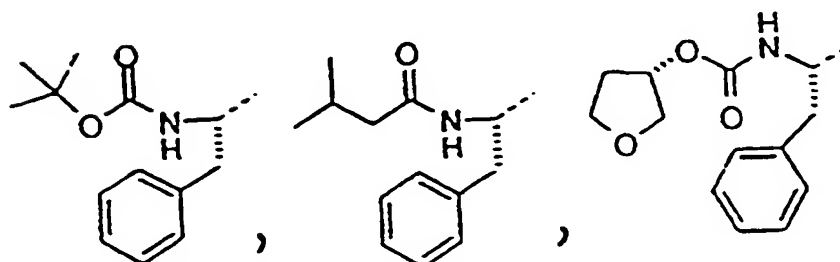
A = alkyl of 1-6 carbon atoms inclusive of linear or branched structures as well as cycloalkyl; and

R = is selected from amino, O-alkyl or N-alkyl

25

B

=



30

or quinoline - Val-Ph -.

Repr sentative values of branch d chain alkyl include
isoamyl, isobutyl and isopropyl. Representative values of N-alkyl

include NMe_2 and O-alkyl includes O-Me. Cycloalkyl may include cyclohexyl or cyclophenyl.

To achieve the objectives of the present invention, we have taken a completely different approach. The X-ray crystal structure is known at 2.2 Å resolution for Ac-Ser-Leu-Asn-Phe-
5 [CHOH-CH₂]-Pro-Ile-Val-OMe known as JG-365 bound to HIVPR (Fitzgerald, P.M. & Springer, J.P., 1991, Ann. Rev. Biophys. Biophys. Chem. 20 299-320). Using molecular modelling techniques to visualize this structure, we noticed that alternate amino acid side
10 chains were adjacent and close enough to be linked together. For example, the proximity of Phe and Leu side chains, suggested the possibility of constructing a small macrocycle as a structural replacement for Leu-Asn-Phe in JG-365. Macrocycles have previously been used (McKervey, M.A. & Ye, T., Tetrahedron 48 , 37 8007-22)
15 in the construction of renin but these were abandoned as drug leads. Unexpectedly, computer models of these macrocycles superimposed precisely upon linear peptides such as JG-365 and hence this provided a novel method of imitating the structural and functional properties of bioactive peptides. Furthermore, by twisting these peptide sequences
20 into cycles, a surprising result was the cycles were resistant to degradative peptidases. A third benefit was as a result of the stability of the cycles, such cycles showed anti-viral activity whereas peptides such as JG-365 do not have anti-viral activity.

FIG. 1 hereinafter shows a model of the structural mimic
25 3 (Table 1 hereinafter - S-isomer) superimposed on the protease-bound conformation of JG-365. While there was a good match for the structures, including the PIV region which was unaffected by the macrocycle substitution, the R-isomer of the chiral alcohol superimposed poorly. The slightly larger N-terminal cycle 13 (Table 2
30 hereinafter) superimposed better than compound 3 referred to in Table 1A hereinafter upon the linear inhibitor where by the benzyclic ring matched the phenylalanine side chain.

Similarly, cycles can be used to mimic the C-terminal portion of linear inhibitors such as compounds 87 to 93 in Table 9 hereinafter. In these cases, a C-terminal cycle represents a structural mimic of Phe-Ile-Leu-Val.

5 By combining the N-terminal cycle with a C-terminal cycle, bicyclic inhibitors can be produced such as compound 111 in Table 11 hereinafter.

FIGS. 11, 12, 13 and 14 show the receptor bound conformations of compounds 3, 87, 134 and 111 (modelled structures overlaid on receptor bound conformation of JG-365 (x-ray structure)).

FIGS. 13-14 demonstrate that cyclic inhibitors of the invention are accurate structural mimics of the peptide JG-365. The inhibitory data indicated that the compounds also exhibited functional mimicry in that they had comparable protease inhibiting activities compared to JG-365.

EXPERIMENTAL

DETERMINATION OF INHIBITION DATA

HIV-1 protease activity was measured at 37°C using a continuous fluorimetric assay based on the method of Toth *et al.*,
20 (Toth *et al.*, 1990, Int. J. Pept. Res. 36 544-550). The substrate, 2-aminobenzoyl-threonine-isoleucine-norleucine-(p-nitro)-phenylalanine-glutamine-arginine-NH₂ (Abz-NF*-6), was synthesised by solid phase peptide synthesis (Kent *et al.*, 1991, In: Peptides 1990: Proceedings
25 of the Twenty-First European Peptide Symposium (Girault, E. and Andrew, D. eds.) pp 172-173, ESCOM, Leiden). Enzyme activity was measured as the increase in fluorescence intensity upon substrate hydrolysis. The change in fluorescence intensity was monitored with a Perkin Elmer LS50B luminescence spectrometer fitted with a
30 thermostatted micro cuvette holder. Measurements were carried out using an excitation wavelength of 320 nm (bandwidth 5 nm) and an emission wavelength of 320 nm (bandwidth 5 nm).

Inhibition assays were routinely carried out at pH 6.5 and $\mu = 0.1$ M using a single substrate concentration. The assay mixtures contained 50 μ M Abz-NF*-6 in 100 mM 2-(N-morpholino)ethanesulphonate buffer, containing 10% (v/v) glycerol and 50 μ g/ml bovine serum albumin, in a total final volume of 400 μ l. Where inhibitors had to be dissolved in dimethyl sulphoxide or other organic solvents, the final concentration of solvent in the control and inhibition assays were kept absolutely constant because of the severe inhibitory effect of some solvents on the enzyme activity (Bergman *et al.*, 1992, 17th Ann. Lorne Conf. on Protein Struct. and Funct. Abstr.). In all assays, the reaction was initiated by the addition of 10 μ l of enzyme solution.

Inhibition was expressed in terms of the IC_{50} value (i.e. the concentration of inhibitor that caused 50% loss of enzyme activity in the standard assay (50 mM Abz-NF*-6, pH 6.5, $\mu = 0.1$ M, 37°C)).

The data obtained by the assays discussed above is reported in Tables 1 to 13.

STRUCTURE-ACTIVITY RELATIONSHIPS FOR CYCLIC INHIBITORS

The class of inhibitors that were investigated in Tables 1 to 13 were based upon natural substrate sequence:- Ser-Leu-Asn-Phe-Pro-Ile-Val.

The first change involving replacement of the scissile amide bond with a "transition-state isostere" gave the known inhibitor JG-365. Inhibitor potencies shown below refer to our assays.

AcSerLeuAsnPhe[*CHOH-CH₂-N]ProIleValOMe

* stereochemistry at this centre	Inhibitor	Potency
S-isomer	IC_{50}	6 nM
R-isomer		44 nM.

This compound is, however, inactive at 100 μ M against HIV in cells. This is likely due to either:-

(a) hydrolysis of peptide bonds inside cells by other

peptidases in route to HIV-1 protease; r

(b) inability to enter cells.

Truncation of the Serin reduces protease inhibition 10-

fold:-

5 AcLeuAsnPhe[*CHOH-CH₂N]ProlleValOMe IC₅₀ 60 nM

Compound 1

We set out to make a range of macrocycles (Tables 1-13 attached) as mimics of this peptide, constraining the LeuAsnPhe component while at the same time providing stability. The cyclization process was expected to protect the peptide amide bonds "invisible" to peptidase enzymes that normally break down peptides. Thus the macrocycles were expected to behave as non-peptide "space-filling" groups.

10 Table 1: Compound 2 and 3 were computer modelled and 3 seemed to overlay reasonably well on compound 1. These were synthesized and the activities indicated that 3 had successfully functionally mimicked compound 1. Interestingly, truncation of the C-terminal end reduced potency (cf. 3 with 5 and 7).

20 The magnitude of this loss of activity is sufficient to account entirely for the inactivity of JG-365 against HIV in cells if cellular peptidases were chopping it up (explanation (a) above).

Next we derivatised the cycle at the N-terminus in an attempt to improve/optimize its activity. Compounds 8-13 (Tables 1 & 2) suggest that expanding the -(CH₂)₃- linker in the cycle with a -(CH₂)₄- or 4 atom spacer did not reduce the potency and modelling suggested that the fit to the enzyme groove was better. Compounds 10 & 11 (Table 1) were thought to offer additional binding from the 2 OH groups to the enzyme's Arg-8 located nearby. This was unsuccessful, since no improvement was observed in potency compared to 3 or 9. However, the stereochemistry of the alcohols needed to be specific to make such interactions and the stereoisomers were not isolated in this work.

Table 2: Compounds 14 & 15 show that the uncyclized version had slightly reduced potency - the reason for cyclizing these molecules was to gain increased stability to hydrolytic enzymes that might "chew" up the inhibitors before they reach their target HIV protease. Surprisingly, there was no dramatic increase in potency for the cyclised inhibitor (e.g. 13) over the uncyclised analogue (e.g. 15), an increase that might have been expected on the basis of conformational entropy arguments - the cycle was preorganised for interacting with the enzyme whereas the more flexible uncyclised inhibitor requires energy to rearrange into a binding conformer.

Compounds 16 and 17 were attempts to increase potency by increasing the size of the macrocyclic ring - activity was instead reduced.

Table 3: Compounds 18-25 were made in the hope of increasing potency due to extension of a group from the macrocyclic ring into a position occupied by Ser of JG-365. (Note that JG-365 was more potent than compound 1). Activities were no better than compound 3. The modelling of the inhibitors suggested that the 3 dangling groups on the left hand side of these cycles in 18-23 have the wrong stereochemistry to mimic the Serine of JG-365. Hence compounds 24 & 25 were made (opposite stereochemistry for $\text{NH}_2\text{CO}(\text{CH}_2)_2$ -substituent on left hand side).

Table 4: These were attempts at remodelling the C-terminus of the cycle (i.e. replacing the Pro-Ile-Val (~PIV) with other groups). Compounds 26 & 27 show that the right hand side has not been as good as PIV since activity is only μM instead of nM. Similarly 28-35 were also less effective. The results for 30-35 did suggest, however, that the R substituent was better for isobutyl than for isopropyl or tertiary butyl.

Table 5: On the other hand, Table 5 shows that Pro-NtBu on the C-terminus is satisfactory with an IC_{50} of 194 nM (compound 38). Note that the cycle is important as 36 & 37 were much less active

than 38 & 39. This was surprising because in 38 the right hand side of the molecule has been truncated by effectively one amino acid (the Val of JG-365). The tertiary butyl side chain that hangs off the proline ring likely fills P2' and there is not much unfilled space in the P2' pocket as shown by the fact that 40 & 41 (were 2 isopropyl groups need more space than 1 tertiary butyl) are much less active.

Compounds 42-45 were designed to keep the t-butyl in P2' and bulk out the P1' position formerly filled by the 5-membered ring of proline. Compound 44 successfully did this and was a landmark compound for this series of inhibitors. Since the cycle is stable, we do not believe that this type of molecule can now be cleaved readily by other peptidases. We did, however, anticipate a problem with cellular uptake since the $\text{LogP}_{\text{o/w}}$ (octanol-water partition co-efficient) was ~ 0.0 , indicating low lipophilicity. Results initially indicated high potency against HIV in cell culture ($\text{EC}_{50} \sim 15 \text{ nM}$). However, this compound was a poor inhibitor of viral replication in cells ($\text{EC}_{50} \sim 5 \mu\text{M}$), a result attributed to the anticipated poor cellular penetration. Attempts have been made to improve cellular permeability by making these compounds more lipophilic without completely losing water solubility.

Table 6: Compounds 46, 47 tested the effect of phenylalanine in the P1' position versus proline used in earlier Tables - there was not a great deal of difference although one feature of the inhibitors which did emerge was that a proline-like P1' substituent goes better with Asn in P2 and a phenylalanine-like P1' substituent goes better with Val in P2.

Compounds 48-53 compare the effect of ring size on inhibitor activity, we find that the larger ring did impart slightly more activity to the inhibitor. Compounds 54 & 55 demonstrated that the isobutyl substituent on the ring was also slightly better than the isopropyl of 48/49. Compounds 56 & 57 tested the effect of inserting a sulfonate into the backbone of the ring in anticipation of interacting

th Arg8/108 of the enzyme.

Table 7: Compounds 58 and 60 use the same macrocycle as before, but report on the usefulness of a sulfonamide in P1' and P2'. Greater activity was obtained with the free amine terminus (60) than the amide (59), although both compounds were potent inhibitors of HIV-1 protease. Compound 60 thus became a lead compound for further development as a potential anti-viral compound. It does indeed show anti-viral activity at concentrations below 1 μ M. Compounds 60-73 were developed as more lipophilic derivatives for evaluation against the virus, with the exception that they might more effectively penetrate cell membranes.

Table 8: Compounds in Table 8 were designed and synthesized to test different P1' and P2' handles on the N-terminal cycle. 74/75, 78/79 and 80/81 contain benzylic groups in P1 and P1' while 76/77 and 82/83 contain a benzylic group on P1 and a proline mimetic in P1'. All R-isomers had values for IC₅₀ 1-1000 nM. The benzimidazole at P2' in 74-77 can hydrogen bond through its NH proton, similar to the hydroxyl group of the indane of 78/79 and the hydroxyl group of the phenylglycinol in 80/81. In compounds 82/83, the benzylic substituent on the pyrazine ring extends into the P3' position affording increased potency.

The same idea exploited for mimicking the N-terminal tripeptide Leu-Asn/Val-Phe with a macrocycle can also be applied to the C-terminal tripeptides Phe-Ile-Val and Pro-Ile-Val.

Table 9: This Table refers to use of a cyclic mimetic of the C-terminal tripeptide Phe-Ile-Val with varying N-terminal substituents. Clearly this cycle leads to potent inhibitors of HIV-1 protease with IC₅₀ values in the range of 1-60 nM. The preferred stereochemistry at the alcohol was R in most cases. The ring size had little effect (cf. 87 versus 88), the hydroxyl group of the transition state isostere was, however, critical for high potency (89 versus 90). The tetrahydrofuran group of 91 was not better as a P2 filler over the t-butyl group in 89

but both were more effective than the aromatic ring in 92 as P2 substituents. Compound 93 containing the quinolin group was a very potent inhibitor of HIV-1 protease and represented a good lead compound for development of anti-viral potential. It is equipotent as a
5 protease inhibitor with the DuPont-Merck compound DM323 but binds by a very different mechanism. It also shows anti-viral activity against HIV in cell culture with an $IC_{50} < 1 \mu M$.

Table 10: Compounds 95-106 were attempts to improve lipophilicity to enhance cellular penetration to further increase anti-viral activity. In these examples, the idea is that the substituent (R) carries a lipophilic tail to add fat solubility without interfering with interactions between the inhibitor and enzyme.
10

Compound 107/108 have slightly enhanced activity due to the better fit and hydrogen-bonding interactions between the Gln side chain and the protease.
15

A logical extension of the concept of mimicking the tripeptides Leu-(Val/Asn)-Phe and Phe-Ile-Val or Pro-Ile-Val is to combine these monocyclic components into a bicyclic hexapeptide mimic. Thus the bicyclic molecules described in Tables 11-13 are potent inhibitors of HIV-1 protease.
20

Table 11: Compound 109 was 100-fold more potent than 110. The R-stereochemistry generally being more favourable for these bicycles which tend to be low nM inhibitors of HIV-1 protease. Compound 111 (IC_{50} 1 nM) was taken as a lead compound for further development.
25 Changes that increase activity include replacing Val with Leu (113/114) and increasing ring size (115/116).

Table 12: Compounds 117-120 compare the effect of an hydroxyproline with a thioproline as P1' substituents of the macrocycle. 121 shows the effect of Asn versus Val in P2 (119) in the presence of a pro-mimic at P1'. 123 has a bulkier piperazine at P1' and this more successfully mimics JG-365, moreover with an Asn instead of Val at P2 even better activity is obtained.
30

Table 13: Compounds 127-130 utilise a phenylglycine at P2 and/or P2'. In our work, we have compared a range of over a dozen peptidic inhibitors of HIV-1 protease by overlaying their known X-ray crystal structures. Remarkably we find that the "consensus" inhibitor is a
 5 bicycle with phenylglycine at positions P2 and P2'. Compound 131/132 with a D-Gln at position P2' were also quite active.

Cell penetration

By developing potent (nM) inhibitors of HIV-1 protease without susceptibility to peptidases (i.e. 'peptide-like' properties), we
 10 envisaged that the ability to enter cells would be related to the partitioning between water and cell membrane.

To predict the partition co-efficients, we used a modelling programme and found that anti-viral activity requires a Log P of 2.5.

We have calculated some partition co-efficients (Log P values) describing solubilities in octanol (o), as being indicative of lipid
 15 or membrane permeability, over water (w) to predict likely uptake of our inhibitors. The following list suggests that the macrocycles are more soluble in octanol than water (Log P > zero) whereas peptides have very little octanol solubility and are very water soluble (Log P is
 20 negative). Our macrocyclic compounds are clearly more water soluble than current HIV-1 protease inhibitors that exhibit anti-viral activity.

To check the importance of Log P, we have calculated it for some non-steroidal anti-inflammatory drugs in the market place and find their numbers are 3-4. Some indications of Log P values for
 25 our macrocycles are given below.

Compound	Log P (o/w)
3	-0.69
18	-0.46
20	-2.03
30 22	-2.80
25	-2.97
34	+0.06 (i.e. octanol solubility = water solubility)

40	+3.61
42	-0.11
44	+2.74
46	+3.25.

5 **HIV-1 PR: INHIBITOR CO-CRYSTALLISATION AND**
 STRUCTURE DETERMINATION

For co-crystallation studies, the inhibitor referred to in FIG. 1 was dissolved in DMSO to give a 130 mM solution. This was mixed in a 1:10 ratio with the Aba-substituted synthetic HIV-1 PR (5 mg/ml in 0.1 M acetate buffer pH 5.5) to give a final inhibitor concentration of inhibitor. The protease:inhibitor mixture crystallised from 40% ammonium sulphate and 0.1 M acetate buffer, pH 5.5.

A rod-shaped crystal measuring 0.4 x 0.1 x 0.1 mm was used for X-ray data measurement of the HIV-1 PR inhibitor complex. Crystallographic data to Å were measured on an imaging plate area detector RAXIS IIC using CuK α X-rays (60kV, 90mA) from an RU-200 rotating anode X-ray generator.

The crystal spacegroup is P2₁2₁2₁, with a unit cell of $a=51.3\text{\AA}$, $b=58.8\text{\AA}$, $c=62.3\text{\AA}$, $\alpha=90^\circ$, $\beta=90^\circ$, $\gamma=90^\circ$ which is isomorphous with several previously reported HIV-1 PR:inhibitor complexes. The merged data (11,924 reflections from 69,201 observations) has an overall R_{mer} of 8.0%, overall I/sigI of 10.7 and represents 97% of all data to 2.06Å. The highest resolution shell (2.06-2.25Å) is 95% complete with I/sigI of 3.7.

The structure of the protease:inhibitor complex was solved by difference Fourier analysis using the protease structure from the complex with JG365 (7hvp.pdb). The inhibitor and solvent atoms were removed from this structure, and the cysteines were modified to Aba residues. The initial R factor, using F_c and Φ_c from this protease model gave an R factor of 31.2% with the measured F_o . The first difference Fourier map ($F_o - F_c$) showed density for the inhibitor at the active site.

The inhibitor was modelled into the density and the structure of the complex refined using X-PLOR. After several rounds of model building, inclusion of solvent, and refinement the current R factor is 17.4% (6 to 2Å) with rms deviations in bond length and
5 bond angles of 0.012Å and 2.073°, respectively.

The structure showed that the inhibitor binds to the protease dimer in much the same way as JG-365 as shown in FIG. 2. There is no observed density for one of the (CH₂)₃ carbons atoms in the ring, indicating that this part of the structure is flexible. Other
10 atoms in this ring may also be poorly localised since the density is weak and during structure minimisation these atoms have refined with high B factors. Nevertheless, the structure clearly shows that the cycle overlays or superimposes closely on JG-365 as it exists in the conformation bound to HIV-1 protease.

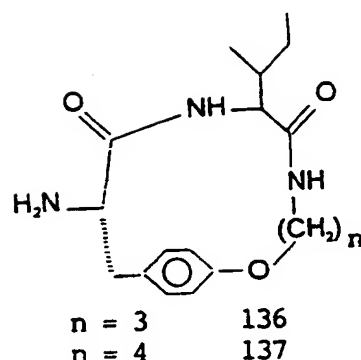
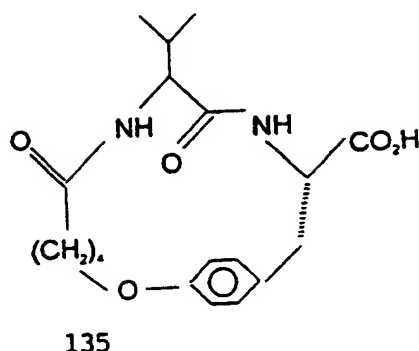
15 Three other inhibitors, i.e. compounds 44, 89 and 123 in addition to compound 3 have also been co-crystallised with HIVPR and their X-ray crystal structures determined (see Table 14). The full X-ray structural data for compounds 3, 44, 89 and 133 has been deposited in the Brookhaven Protein Data Bank in the United States.

20 STABILITY OF MACROCYCLES

Evidence of macrocycle stability is shown in FIGS. 3-9 which show mass spectra of macrocycles after exposure to the gastric peptidases pepsin 3a, gastrixin and cathepsin D. This data demonstrates that the macrocycles are proteolytically stable *in vitro*.

25 In this procedure, the relevant compounds were exposed to peptidases for 1 hour at 37°C and then assayed by electrospray mass spectroscopy. Molecular weights were determined from the data indicating that the macrocycles remained intact after contact with the peptidases.

5



10 These above cycles were also evaluated for stability in 3 M HCl and human blood.

Stability in hydrochloric acid

15 The above cycles were dissolved in 3 M HCl and heated to ~ 45°C. The reactions were monitored daily for 4 days by mass spectrometry. There was no hydrolysis or degradation of the cycles over this time.

Stability in human blood

20 Human blood (5 ml) was allowed to clot for 1 hour before centrifuging (5 min). Test compound (200 µg) was assayed in duplicate by adding 450 µl saline or serum, vortexing and incubating (1 hr, 37°C). After adding acetonitrile (1 ml, 4°C), vortexing (30 s) and centrifuging (5 min, 4°C), the supernatant was removed. After freezing in liquid nitrogen, lyophilizing to dryness, resuspending in 100 µl buffer, the products were analysed by HPLC and electrospray mass spectrometry. No degradation products were observed, only the unchanged cyclic compounds were detected.

25

ASSAY FOR ANTI-VIRAL ACTIVITY

30 Method: Target cells (PHA-stimulated cord blood mononuclear cells) were washed free of PHA and inoculated with HIV-1 (strain TC354) at a multiplicity of infection of 1, in the presence of 20 µg/ml DEAE-dextran for 30 mins. Cells were washed to remove unbound virus and residual DEAE-dextran. 10⁵ cells were added to each well of 96 well plate, solutions of test compounds were added, each tested in

triplicate. Dilutions were prepared in RPMI/1640. Tissue culture medium (RPMI/1640 buffer, 10% FCS, 20 U/ml rU-2) was added to a final volume of 200 μ l. Reverse transcriptase activity was assayed four days post inoculation, RT activity > 30 cpm was considered positive for HIV-1 replication.

Representative test compounds subjected to anti-viral testing included 44, 68 and 93. These compounds inhibited replication of the virus in the above assay with effective concentrations of 1 μ M, 100 nM and 100 μ M respectively.

10

SYNTHESIS OF CYCLIC INHIBITORS

In relation to preparation of the protease inhibitors of the present invention, suitable methods of preparation of these compounds are described in Schemes 1, 2, 3 and 4 attached herewith. Scheme 1 is suitable for preparation of compounds of structure (i) where Z is peptide, Scheme 2 is suitable for preparation of compounds of structure (i) where Z is non-peptide and Scheme 3 and 4 are suitable for preparation of compounds of structure (ii). Structure (iii) may be made by a combination of Schemes 1, 2 and 3 or closely related synthetic methods.

20

Scheme 1 depicts a synthesis of macrocycle 2 and 3 which, with minor modifications was also used for the synthesis of derivatives 4-25. The direct alkylation of Boc-tyrosine 1 with ethyl 4-bromobutyrate gave exclusively the O-alkylated tyrosine 1a. This was converted by an established method (McKervey, M.A. & Ye, T., Tetrahedron, 48, 37, 8007-22) to the diazoketone, and subsequently to the bromomethyl ketone 1b with HBr. Tripeptide 4 was assembled on MBHA resin using HBTU, alkylated with the bromomethyl ketone (1b), followed by reduction with sodium borohydride to give the hydroxyethylamine isostere 5. After introducing Asn, the peptide was cleaved from resin (HF), de-esterified with NaOH, and the mixture of diastereomers (6) was purified by reverse phase HPLC in 54% overall yield. Dilute (mM) solutions of (6) were cyclized intramolecularly

30

using BOP to give 2 and 3. The diastereomers were separated by RP-HPLC to give pure 2, S (21%) and 2, R (28%) which were characterized by ion spray mass spectrometry (M+H, 688). The stereochemistry of the alcohol was verified for the S-isomer by X-ray crystallography of the inhibitor-bound complex 3-HIVPR (The three dimensional crystal structure was determined for a complex of 7a with HIVPR (Wickramsinghe, W. & Martin, J., University of Queensland, unpublished work) to a resolution of 2.1Å. This structure identified the isomer as 7a,S and showed almost identical structural overlay as the model on the crystal structure of JG-365 bound to HIVPR.) The two chiral centres in the macrocycles 2 and 3 were thus derived from simple L-amino acids.

Also attached herewith is details of Schemes 2 and 3 which describes the synthesis of other N-terminal cycles and Schemes 3 and 4 describe alternative syntheses of C-terminal cyclic inhibitors.

The bicycles (Tables 11-14) were prepared by general synthesis involving coupling of the C-terminal cycle (compound 4 in Scheme 3) with (compound bottom LHS) compound Z from Scheme 4.

SYNTHESIS OF COMPOUNDS COMPRISING HYDROLYTICALLY STABLE CYCLIC MIMETICS OF TRIPEPTIDES LEU-ASN-PHE AND PHE-ILE-VAL

General methods

All materials were obtained commercially as reagent grade unless otherwise stated. Melting points were determined on a Reichert hot stage apparatus and are uncorrected. ¹H NMR spectra were recorded on a Bruker ARX 500 MHz spectrometer using D₂O as internal standard for water-soluble samples or TMS for spectra recorded in CDCl₃. Proton assignments were determined by 2D COSY & TOCSY experiments. ¹³C NMR spectra were measured on a Varian Gemini 300 NMR spectrometer and chemical shifts are reported in ppm relative to CD₃OH, EtOH or CDCl₃. Preparative scale reverse

phase HPLC separations were performed on Waters Delta-Pak PrepPak C₁₈ 40 mm x 100 mm cartridges (100 Å); analytical reverse phase HPLC on Waters Delta-Pak Radial-Pak C₁₈ 8 mm x 100 mm cartridges (100 Å); using gradient mixtures of water/0.1% TFA and water
 5 10%/acetonitrile 90%/TFA 0.1%.

Mass spectra were obtained on a triple quadrupole mass spectrometer (PE SCIEX API III) equipped with an Ionspray (pneumatically assisted electrospray) (Bruins *et al.*, 1987, Anal. Chem. 59 2642) atmospheric pressure ionisation source (ISMS).
 10 Solutions of compounds in 9:1 acetonitrile/0.1% aqueous trifluoroacetic acid were injected by syringe infusion pump at μ M-pM concentrations and flow rates of 2-5 μ l/minute into the spectrometer. Molecular ions, $\{[M + nH]^n\}^+$, were generated by the ion evaporation process (Iribane, J.V. & Thomson, B.A., 1976, 64 2287) and
 15 focussed into the analyser of the mass spectrometer through a 100 mm sampling orifice. Full scan data was acquired by scanning quadrupole-1 from m/z 100-900 with a scan step of 0.1 dalton and a dwell time of 2 msec. Accurate mass determinations were performed on a KRATOS MS25 mass spectrometer using Electron Impact
 20 ionisation.

Abbreviations

LNF = Leu-Asn-Phe;
 PIV = Pro-Ile-Val;
 DIPEA = diisopropylethylamine;
 25 MBHA = p-Methylbenzhydrylamine resin. HCl, 0.79meq/g;
 MF = dimethylformamide;
 BOP = [Benzotriazol-1-yl-oxy-tris(dimethylamino) phosphonium]hexafluorophosphate;
 HBTU = O-Benzotriazole N',N',N',N'-tetramethyluronium
 30 hexafluorophosphate;
 TFA = Trifluoroacetic acid.
 Ethyl 4-[4'-(2-(t-butoxy carbonylamino)-2-oxoethyl)phenoxy]

butan ate (Z)

To a suspension of NaH (1.2 g, 50 mmol) in dry THF (80 ml) was slowly added Boc-tyrosine (3.5 g, 12.46 mmol), under an argon atmosphere. The solution was stirred at room temperature for 5 minutes, after which ethyl 4-bromobutanoate (7.28 g, 37.35 mmol) was added in one portion and the suspension heated at reflux for 16 hrs. A further 3eq. of both NaH and ethyl 4-bromobutanoate was added and heating was continued for 5 hrs. Solvent was removed under reduced pressure, the residue dissolved in water (100 ml), and the basic solution was extracted with diethyl ether (3 x 40 ml) to remove unreacted ethyl 4-bromobutanoate and ethyl cyclopropanecarboxylate side product. The aqueous layer was acidified (pH = 2) with 1 M KHSO₄ and extracted with ethyl acetate (4 x 50 ml). The organic phase was dried and evaporated to give the title compound (4.2 g, 91%) as a colourless oil. Although used directly for subsequent reactions, a small amount was purified by reverse phase HPLC (70:30, water/acetonitrile/0.1% TFA) to give a colourless oil. ¹H NMR (CDCl₃) δ; AA'BB' system: 7.08 (d, *J*_{AB} + *J*_{AB'}, 2H, 8.61Hz, ArH ortho to CH₂), 6.81 (d, *J*_{AB} + *J*_{AB'}, 8.61Hz, 2H, ArH ortho to O), 6.60 (br. s., 1H, COOH), 4.94 (d, 7.6Hz, 1H, NH), 4.55 (m, 1H, Tyr αCH), 4.14 (q, *J* = 7.2Hz, 2H, OCH₂CH₃), 3.98 (t, *J* = 6.1Hz, 2H, O-CH₂-CH₂-CH₂), 3.14 (dd, *J* = 12.8, 5.1Hz, 1H, Tyr βCH), 3.02 (dd, *J* = 12.8, 6.0Hz, 1H, Tyr βCH), 2.50 (t, *J* = 7.3Hz, 2H, CH₂COOEt), 2.09 (m, 2H, CH₂-CH₂-CH₂), 1.42 (s, 9H t-butyl), 1.26 (t, *J* = 7.2 Hz, 3H, OCH₂CH₃). ¹³C NMR (CDCl₃) δ 14.19 (OCH₂CH₃), 24.62 (CH₂-CH₂-CH₂), 28.12 (t-butyl CH₃), 30.74 (CH₂COOEt), 36.88 (Tyr βCH), 54.35 (Tyr αCH), 60.47 (O-CH₂), 66.66 (OCH₂CH₃), 80.18 (t-butyl), 114.44 (ArC, ortho to CH₂), 127.78 (Ar-CH₂), 130.37 (ArC, ortho to O), 155.36 (ArC-O), 157.93 (C=O, carbamate), 173.35 (C=O, ethyl ester), 176.25 (C=O, COOH). ISMS 413(M+NH₄), 396(M + H), 340(M + H-isobutene), 296(M+H-BOC). HRMS calcd for C₂₀H₂₉NO₇, 395.1944; found

395.1943.

(S)-3-(t-Butoxycarbonylamino)-1-diazo-4-(4'-[3-carboethoxy]propyloxy)phenyl-2-butanone (Y)

To a solution of alkylated Boc-tyrosine (Z) (4 g, 10.1 mmol) in dry THF (50 ml) was added N-methyl piperidine (1.58 ml, 12.1 mmol). The solution was cooled to -10°C under an atmosphere of dry nitrogen and ethyl chloroformate (1.2 g, 1.05 ml, 11.1 mmol) was added in one portion. The solution was stirred for 10 mins during which N-methyl piperidine hydrochloride precipitated. An ethereal solution of diazomethane (excess) was added dropwise to this suspension over 30 mins at -5°C. The reaction mixture was allowed to warm to room temperature over 1 hr after which a slow stream of nitrogen was bubbled through the solution for 15 mins to remove any unreacted diazomethane. The solution was diluted with ether (150 ml) and washed with water (3 x 100 ml), saturated NaHCO₃ (2 x 100 ml) and brine (1 x 100 ml). The organic phase was dried with MgSO₄ and evaporated *in vacuo* to give the title compound as a light yellow oil (3.2 g, 75%). While sufficiently pure for synthetic purposes, a small amount was purified by radial chromatography (ethyl acetate/light petroleum, 1:3) and subsequently recrystallised from hexane to give colourless needles, m.p. 73-75°C. ¹H NMR (CDCl₃) δ AA'BB' system: 7.08 (d, $J_{AB} + J_{AB'}$ = 8.52 Hz, 2H, ArH ortho to CH₂), 6.82 (d, $J_{AB} + J_{AB'}$ = 8.52 Hz, 2H, ArH ortho to O), 5.21 (s, 1H, CHN₂), 5.12 (d, J = 8.1 Hz, 1H, NH), 4.36 (m, 1H, Tyr αC-H), 4.15 (q, J = 7.2 Hz, 2H, OCH₂CH₃), 3.98 (t, J = 6.1 Hz, 2H, O-CH₂), 2.95 (d, J = 6.4 Hz, 2H, Tyr βCH), 2.51 (t, J = 7.3 Hz, 2H, CH₂COOEt), 2.10 (m, 2H, CH₂-CH₂-CH₂), 1.41 (s, 9H, t-butyl), 1.26 (t, J = 7.2 Hz, 3H, OCH₂CH₃). ¹³C NMR (CDCl₃) δ 14.22 (OCH₂CH₃); 24.63 (CH₂-CH₂-CH₂); 28.28 (t-butyl CH₃); 30.79 (CH₂COOEt); 37.73 (Tyr βCH); 54.41 (Tyr αC-H); 58.50 (CHN₂); 60.43 (O-CH₂); 66.69 (OCH₂CH₃); 80.07 (t-butyl); 114.58 (ArC, ortho to CH₂); 128.21 (Ar-CH₂); 130.34 (ArC, ortho to O); 155.13 (ArC-O); 157.85 (C=O, carbamate);

173.20 (C=O, ethyl ester); 193.13 (diazoketone). ISMS: 437(M+NH₄⁺); 420(M+H); 336(M+H-isobutene-N₂); 292(M+H-BOC-N₂); Anal. calcd for C₂₁H₂₉N₃O₆ C, 60.13%; H, 6.97%; N, 10.02%; Found: C, 60.16%; H, 6.97%; N, 9.85%.

5 **1-Bromo-(S)-3-(t-butoxycarbonylamino)-4-(4'-[3'-carboethoxy]propyloxy) phenyl-2-butanone (X)**

A saturated solution of HBr in ethyl acetate was diluted 1:9 with ethyl acetate and added in 1 ml aliquots to a cold solution (0°C) of the diazoketone (Y) (1.75 g, 4.18 mmol) in ethyl acetate (40 ml). The progress of the reaction was followed by thin layer chromatography. On completion the reaction mixture was washed with a 0.1 M NaHSO₄ (2 x 50 ml), saturated NaHCO₃ (2 x 40 ml) and brine (1 x 40 ml). The organic phase was dried with MgSO₄ and evaporated to dryness to give the title compound as a colourless solid (1.5 g, 75%). While sufficiently pure for synthetic purposes, a small amount was purified by radial chromatography (ethyl acetate/light petroleum, 1:3) and subsequently recrystallised from hexane/dichloromethane to give colourless needles, m.p. 92-94°C. ¹H NMR (CDCl₃) δ AA'BB' system: 7.07 (d, J_{AB}+J_{AB'} = 8.60Hz, 2H, ArH ortho to CH₂), 6.83 (d, J_{AB}+J_{AB'} = 8.60Hz, 2H, ArH ortho to O); 5.03 (d, J = 7.2Hz, 1H, -NH); 4.67 (m, 1H, Tyr αC-H), 4.15 (q, J = 7.2Hz, 2H, OCH₂CH₃), 3.99 (t, J = 6.1Hz, 2H, O-CH₂), 3.97 (d, J = 13.9Hz, 1H, -CHBr), 3.83 (d, J = 13.9Hz, 1H, -CHBr), 2.95 (m, 2H, Tyr βCH), 2.51 (t, J = 7.3Hz, 2H, CH₂COOEt), 2.10 (m, 2H, CH₂-CH₂-CH₂), 1.42 (s, 9H, t-butyl), 1.26 (t, J = 7.2 Hz, 3H, OCH₂CH₃). ¹³C NMR (CDCl₃) δ 14.15 (OCH₂CH₃); 24.54 (CH₂-CH₂-CH₂); 28.19 (t-butyl CH₃); 30.71 (-CH₂COOEt); 33.37 (-CH₂Br), 37.00 (Tyr βCH); 58.61 (Tyr αCH); 58.50 (CHN₂); 60.37 (O-CH₂); 66.66 (OCH₂CH₃); 80.34 (t-butyl); 114.77 (ArC, ortho to CH₂); 127.58 (Ar-CH₂); 130.09 (ArC, ortho to O); 155.15 (ArC-O); 157.99 (C=O, carbamate); 173.12 (C=O, ethyl ester); 200.95 (C(O)CH₂Br). ISMS: 491/489 (M+NH₄,1:1); 474/472 (M+H,1:1); 418/416 (M+H-isobutene,1:1);

374/372 (M+H-BOC, 1:1): Anal. calcd for $C_{21}H_{30}BrNO_6$ C, 53.40%; H, 6.40%; N, 2.97%, Found: C, 53.37%; H, 6.30%; N, 2.84%.

[(2S) and (2R)] (S)-3-((S)-AsparaginyI)amino-4-(4'-[3'-carboethoxy]propyl oxy)-phenyl-1-((S)-N-prolyl-(S)-isoleucyl-(S)-valine amide)butan-2-ol (W)

Assembly of the uncyclised peptidomimetic (W) was accomplished by solid phase peptide synthesis (Alewood *et al.*, 1992, Teett. Lett. 33 977-80). MBHA resin (2.15 g, 2 mmol, S.V. = 0.93 meq/g) was shaken with DIPEA (0.7 ml, 4.0 mmol) in DMF (12 ml) for 2 mins. The resin was filtered and to it was added a solution of Boc-valine (1.74 g, 8 mmol), HBTU (0.5 M solution in DMF, 16 ml, 8 mmol) and DIPEA (2.75 ml, 16 mmol). The resin was shaken with this solution for 10 mins and reaction was monitored by the negative ninhydrin test, which indicated that a 99.65% coupling was achieved. The resin was washed with DMF, treated with TFA (2 x 10 ml, 1 min each) to give the deprotected valine on resin, and the procedure was repeated for Boc-isoleucine (99.99%) and Boc-proline (99.96%). The hydroxyethylamine isostere was introduced by shaking the resin with a solution of the ketobromide (X) (2.36 g, 5 mmol) and DIPEA (1.72 ml, 10 mmol) in DMF (16 ml) for 30 mins. The resultant ketone was reduced by shaking the resin, at room temperature for 1 hr, with sodium borohydride (0.6 g, 16.2 mmol) in THF (16 ml). Boc-asn was coupled to the peptide using the same procedure descibed above (99.66%). The weight of dried resin was 3.8 g (theoretical wt. = 3.85 g). The peptide was cleaved from resin (0.8 g, 0.42 mmol) with HF, lyophilised and treated with a 0.1 M ammonium carbonate solution at room temperature for 15 mins, and again lyophilised to give 279 mg of a powder consisting of a diastereomeric mixture of peptides, ISMS 734(M + H). The diastereomeric mixture was d esterified without further purification by dissolving in a mixtur of wat r (3 ml) and 0.57 M NaOH solution (2.16 ml, 12.3 mmol). Th suspensi n was made homogen ous by the gradual addition of THF

and the resulting solution stirred at room temperature for 30 mins. The mixture was neutralised with 1 M HCl to pH = 7 and the solvent evaporated *in vacuo*. Purification by HPLC (gradient; water 0.1%TFA to 50:50 water/ acetonitrile 0.1% TFA over 75 mins) gave a

5 diastereomeric mixture of (W) (156 mg, 53% overall yield from beginning of the solid phase synthesis). ISMS; 706 (M + H).

[2(S),11'(S),8'(S)]-2-[11'-[6',9'-dioxo-8'-(ethanamide)-2'-oxa-7',10'-diazabicyclo[11.2.2]heptadeca-13',15',16'-triene]]-1-(N-(S)-prolyl-(S)-isoleucyl-(S)-valine amide)ethan-2-ol (V)

10 1a S. The diastereomeric mixture (W) (66 mg, 0.093 mmol) was dissolved in DMF (800 ml, C = 1.16×10^{-4} M), BOP reagent (61.6 mg, 0.14 mmol) and DIPEA (0.1 ml, 0.58 mmol) were added, and the solution stirred at room temperature for 1 hr. The solvent was evaporated *in vacuo* and the residue redissolved in

15 distilled water (20 ml). Insoluble precipitate was filtered from the solution and the diastereomeric mixture was purified by reverse phase HPLC (gradient; water 0.1%TFA to 50:50 (water/ 0.1%TFA)/(water 10%/acetonitrile 90%/TFA 0.1%) over 75 mins) to give a pure sample of both 1a R (18 mg, 28%,) and 1a S (13 mg, 21%,) as white

20 powders, after lyophilisation. The two diastereomers were pure by analytical HPLC analysis (gradient; water 0.1%TFA to 50:50 (water/0.1%TFA)/(water 10%/acetonitrile 90% /TFA 0.1%) over 50 mins), 1a S *rt* = 39.9 min; 1a R *rt* = 42.2 min. ¹H NMR 1aS (H₂O/D₂O, 8:2, 313 K) δ 8.69 (br.s., 1H, Ile-NH), 8.22 (d, J = 7.23Hz, 1H, Val-NH), 7.71 (d, J = 9.8Hz, 1H, 10'-NH), 7.65 (br.s., 1H, Val-1°amide), 7.47 (br.s., 1H, Asn-1°amide), 7.21 (d, J = 8.8Hz, 1H, Asn-NH), 7.20 (dd, J = 2.1, 8.4Hz, 1H, H17'), 7.15 (dd, J = 2.2, 8.4Hz, 1H, H14'), 7.09 (br.s., 1H, Val-1°amide), 7.00 (dd, J = 2.7, 8.4Hz, 1H, H16'), 6.95 (dd, J = 2.7, 8.4Hz, 1H, H15'); 6.72 (br.s., 1H, Asn-1°amide), 4.42-4.48 (m, 1H, H-3'), 4.29-4.38 (m, 2H, Asn- α CH & H-3'), 4.10-4.16 (m, 2H, Pro- α CH & H-2), 3.8 (m, 1H, Pro- δ CH), 3.15-3.28 (m, 3H, Pro δ CH & H-1), 3.12 (dd, J_{H12'-H12'}

25 30

= 3.5Hz, $J_{H_{12'}-H_{11'}} = 5.6\text{Hz}$, 1H, H-12'), 2.78 (dd, $J_{H_{12'}-H_{11'}} = 13.5\text{Hz}$, $J_{H_{12'}-H_{12'}} = 13.5\text{Hz}$, 1H, H-12'); 0.94 (t, 3H, $J = 7.4\text{Hz}$, Ile- δCH_3), 4.22-4.29 (m, 1H, H-11'), 4.19 (m, 1H, Val- αCH), 2.55 (m, 1H, Pro- βCH), 2.42-2.51 (m, 3H, Asn- βCH_2 & H-5'), 2.29 (ddd, $J_{H_{5'}-H_{5'}} = 16.2\text{Hz}$, $J_{H_{5'}-H_{4'}} = 7.6\text{Hz}$, $J_{H_{5'}-H_{4'}} = 3.6\text{Hz}$, 1H, H-5'), 2.19 (m, 1H, Pro- γCH), 1.98-2.14 (m, 5H, Val- βCH , Pro- βCH , Pro- γCH , Ile- γCH & H-4'), 1.89-1.98 (m, 1H, Ile- βCH), 1.51-1.61 (m, 1H, Ile- γCH), 1.20-1.30 (m, 1H, Ile- γCH), 1.02 (d, $J = 6.8\text{Hz}$, 6H, Val- γCH_3), 0.98 (d, $J = 6.8\text{Hz}$, 3H, Ile- γCH_3). ^{13}C NMR: ($\text{H}_2\text{O}/\text{D}_2\text{O}$, 8:2, ref: EtOH); 10.59, 15.06, 18.21, 18.69, 23.08, 24.25, 24.98, 30.04, 30.23, 31.50, 35.63, 36.51, 38.86, 50.51, 53.84, 55.49, 58.19, 59.18, 59.74, 68.42, 68.78, 78.50, 114.98, 117.23, 117.83, 129.45, 131.27, 132.44, 156.90, 158.00, 171.93, 173.71, 174.50, 176.13. ISMS: 688(M+H).

15 3-[N-t-Butoxycarbonyl-(S)-isoleucinyl]amino-1-bromopropane (U)

To a solution of Boc-isoleucine hemihydrate (2.4 g, 10 mmol) and BOP reagent (4.42 g, 10 mmol) in dry THF (50 ml) was added DIPEA (1.29 g, 10 mmol) and the solution stirred for five mins. 3-Bromopropylamine.HBr (2.4 g, 11 mmol) and DIPEA (1.56 g, 12 mmol) were then added to the solution. After 30 mins, the solvent was removed under vacuum, the residue redissolved in ethyl acetate (100 ml) and thoroughly washed with 1 M hydrochloric acid (4 x 50 ml), saturated sodium bicarbonate (2 x 50 ml), brine (1 x 50 ml), and dried over sodium sulphate. Purification by column chromatography (silica; 40% ethyl acetate in hexane ($R_f = 0.8$)) provided the title compound (3.3 g, 94%) as a white solid. ^1H NMR (CDCl_3): δ 6.50 (br.s, 1H, NH), 5.15 (br.s, 1H, Ile-NH), 3.85 (m, 1H, Ile- αCH), 3.40 (m, 4H, NCH_2 , BrCH_2), 2.05 (m, 2H, CH_2), 1.85 (m, 1H, Ile- βCH), 1.40 (m, 10H, Ile- γCH & $(\text{CH}_3)_3$), 1.10 (m, 1H, Ile- γCH), 0.90 (d, $J = 6.82\text{ Hz}$, 3H, Ile- γCH_3), 0.86 (t, $J = 7.73\text{ Hz}$, 3H, Ile- δCH_3); ISMS (M+H) 351/353.

3-[N-t-Butoxycarbonyl-(S)-tyrosinyl-(S)-isoleucinyl]amino-1-

br m propan (T)

Compound (U) (3.51 g, 10 mmol) was dissolved in a solution of 25% TFA in DCM (10 ml) and stirred at room temperature for 30 mins. The solution was evaporated to dryness *in vacuo* and the residue evaporated from DCM three more times to remove residual traces of TFA. This residue was coupled to Boc-tyrosine (2.8 g, 10 mmol) using the procedure described for the synthesis of compound (U). The resultant product was purified by column chromatography (silica; 50% ethyl acetate in hexane (R_f = 0.19)) as a colourless powder (4.9 g, 96%), mp 96-100°C. ^1H NMR (CDCl_3): δ 6.7-7.1 (m, 6H, ArH, Ile-NH, NH), 5.15 (d, J = 8.2 Hz, 1H, Tyr- NH), 4.30 (m, 1H, αCH), 4.2 (m, 1H, αCH), 3.35-3.50 (m, 1H, NCH), 3.40 (t, J = 6.0 Hz, 2H, CH_2Br), 3.25 (m, 1H, NCH), 3.01 (dd, J = 6.1, 13.6 Hz, 1H, Tyr- βCH), 2.93 (dd, J = 6.1, 13.6 Hz, 1H, Tyr- βCH), 2.05 (m, 2H, CH_2), 1.9 (m, 1H, Ile- βCH), 1.4 (m, 10H, Ile- γCH , $(\text{CH}_3)_3$), 1.05 (m, 1H, Ile- γCH), 0.89 (d, J = 6.32 Hz, 3H, Ile- γCH_3), 0.83 (t, J = 7.37 Hz, 3H, Ile- δCH_3). ISMS 514/516 ($\text{M}+\text{H}$). HRMS calcd for $\text{C}_{23}\text{H}_{35}\text{N}_3\text{O}_5(\text{M}-\text{HBr})$, 433.2577; found 433.2573.

[11(S),8(S)]-11-(t-Butoxycarbonylamino)-7,10-dioxo-8-(1-methylpropyl)-2-oxa-6,9-diazabicyclo[11.2.2]heptadeca-13,15,16-triene (S)

The dipeptide (T) (1 g, 1.9 mmol) was added to a solution of sodium methoxide (0.1 g, 1.9 mmol) in methanol (40 ml) and stirred at room temperature for 96 hrs. The solution was evaporated to dryness and diluted with ethyl acetate (50 ml) washed with 1 M hydrochloric acid (1 x 25 ml), saturated sodium bicarbonate (1 x 25 ml), brine (1 x 25 ml). The organic phase dried and the solvent removed. The crude product was purified by column chromatography (silica; 50% ethyl acetate in hexane (R_f = 0.17)) providing the title compound (0.63 g, 75%) as a colorless solid m.p. 248-252°C. ^1H NMR (CDCl_3) δ 7.85 (m, 1H, NH-6), 7.16 (d, J = 8.75Hz, 1H, ArH), 6.97 (d, 1H, J = 7.50Hz, Ile-NH), 6.94 (d, J =

7.50Hz, 1H, Tyr-NH), 6.83-6.90 (m, 3H, ArH), 4.38 (dt, $J_{H3-H3} = 12.5\text{Hz}$ $J_{H3-H4} = 5.00\text{Hz}$, 1H, H-3), 4.22 (m, 1H, H-3), 4.14 (m, 1H, H-11), 3.45-3.55 (m, 2H, H-8 & H-5), 3.08 (dd, $J_{H12-H12} = 13.1\text{Hz}$ $J_{H11-H12} = 6.25\text{Hz}$, 1H, H-12), 2.84 (m, 1H, H-5), 2.59 (m, 1H, H-12),
 5 2.19 (m, 1H, H-4), 1.79 (m, 1H, H-4), 1.57 (m, 1H, Ile- β CH), 1.43-1.49 (m, 10H, Ile- γ CH & t-Butyl), 0.98 (m, 1H, Ile- γ CH), 0.83 (d, $J = 7.5\text{Hz}$, 3H, Ile- γ CH₃), 0.76 (d, $J = 6.3\text{Hz}$, 3H, Ile- δ CH₃). ISMS 434 (M + H). HRMS calcd for C₂₃H₃₅N₃O₅, 433.2577; found 433.2584.

10 **Methyl-(S)-2-(t-butoxycarbonyl-(S)-valinyl)amino-3-phenyl propanoate (R)**

The title compound was prepared by coupling Boc-valine (2.17 g, 10 mmol) and phenylalanine methyl ester.HCl (2.3 g, 11 mmol) with BOP reagent according to the procedure described for (S).
 15 Purification of the crude residue by column chromatography (silica; 50% ethyl acetate in hexane) gave (R) (3.66 g, 97%) as a white solid, mp 103-106°C. ¹H NMR (CDCl₃): δ 7.1-7.35 (m, 5H, ArH), 6.35 (d, $J = 8.33\text{ Hz}$, 1H, Phe-NH), 5.05 (d, $J = 7.7\text{ Hz}$, 1H, Boc-NH), 4.85 (ddd, $J = 6.3, 6.3, 8.33\text{ Hz}$, 1H, Phe- α CH), 3.9 (dd, $J = 7.7, 7.7$
 20 Hz, 1H, Val- α CH), 3.7 (s, 3H, OCH₃), 3.15 (dd, $J = 6.3, 14.1\text{ Hz}$, 1H, Phe- β CH), 3.08 (dd, $J = 6.3, 14.1\text{ Hz}$, 1H, Phe- β CH), 2.1 (m, 1H, Val- β CH), 1.45 (s, 9H, (CH₃)₃), 0.9 (d, $J = 5.0\text{ Hz}$, 3H, Val- γ CH₃), 0.85 (d, $J = 5.0\text{ Hz}$, 3H, Val- γ CH₃). ISMS 379 (M + H). HRMS calcd for C₂₀H₃₀N₂O₅, 378.2155; found 378.2151.

25 **Methyl-(S)-2-(t-butoxycarbonyl-(S)-leucinyI-(S)-valinyl)amino-3-phenyl propanoate (Q)**

The dipeptide (R) (3.5 g, 9.2 mmol) was deprotected at the N-terminus with a solution of 25% TFA in DCM (10 ml) and the resultant amine coupled to Boc-leucine (2.49 g, 10 mmol) using the
 30 same procedure described for (U) above. Column chromatography (silica; 50% ethyl acetate in hexane) provided (Q) (4.3 g, 95%) as a white solid, mp 133-134°C. ¹H NMR (CDCl₃): δ 7.00-7.30 (m, 5H,

ArH), 6.70 (d, $J = 9.30$ Hz, 1H, Val-NH), 6.55 (d, $J = 8.14$ Hz, 1H, Ph -NH), 5.00 (d, $J = 8.20$ Hz, 1H, Boc1 u-NH), 4.85 (m, 1H, Phe- α CH), 4.27 (m, 1H, α CH), 4.10 (m, 1H, α CH), 3.50 (s, 3H, OCH₃), 3.10 (m, 2H, Phe- β CH₂), 2.10 (m, 1H, Val- β CH), 1.35-1.70 (m, 12H, (CH₃)₃, Leu- β CH₂, Leu- γ CH), 0.70-1.00 (m, 12H, Val- γ CH₃, Leu- δ CH₃). ISMS 492 (M + H). HRMS calcd for C₂₆H₄₁N₃O₆, 491.2995; found 491.2996.

Methyl-(S)-2-(acetyl-(S)-leuciny1-(S)-valiny1)amino-3-phenyl propanoate (P)

The tripeptide (P) was prepared by deprotection of (Q) (4 g, 8.1 mmol) with 25% TFA in DCM. The solvent and excess TFA was evaporated before diluting the residue with THF (100 ml). To the solution was added DIPEA (approx. 10 ml, excess) and DMAP (1 mole %). The resulting solution was then cooled to 0°C in an ice-bath before adding acetic anhydride (5 equivalents). The mixture was warmed to room temperature and stirred for a further 30 mins. The solvent was removed and the residue redissolved in ethyl acetate (200 ml). The organic phase was washed with 1M hydrochloric acid (1 x 50 ml), saturated sodium bicarbonate (1 x 50 ml), brine (1 x 50 ml), dried and the solvent evaporated. The product was recrystallised from ethyl acetate to provide the title compound (3.45 g, 99%) as a white solid mp 216-217°C. ¹H NMR (CDCl₃): δ 7.10-7.30 (m, 6H, ArH, NH), 7.0 (d, $J = 9.0$ Hz, 1H, NH), 6.5 (d, $J = 9.1$ Hz, 1H, NH), 4.9 (m, 1H, Phe- α CH), 4.60 (m, 1H, Leu- α CH), 4.45 (m, 1H, Val- α CH), 3.70 (s, 3H, O-CH₃), 3.10 (m, 2H, Phe- β CH₂), 2.10 (m, 1H, Val- β CH), 2.00 (s, 3H, CH₃C(O)), 1.45-1.70 (m, 3H, Leu- γ CH & Leu-CH₂), 0.90-1.05 (m, 12H, Val- γ CH₃ & Leu- δ CH₃). ¹³C NMR: (CDCl₃) 18.18, 18.99, 22.32, 22.92, 22.99, 24.82, 31.14, 35.07, 41.42, 51.74, 52.23, 52.26, 58.41, 127.11, 128.57, 129.17, 135.82, 170.20, 170.80, 171.84, 172.35. ISMS: 434 (M + H). HRMS calcd for C₂₃H₃₅N₃O₅, 433.2577; found 433.2576.

(S)-2-(Acetyl-(S)-leuciny1-(S)-valiny1)amino-1-diaz -3-phenyl butan-2-

one (O)

The tripeptide (O) (1 g, 2.3 mmol) was de sterified by dissolving in a mixture of dioxan (50 ml) and 1 M sodium hydroxide (10 ml), stirring at room temperature for 30 mins, before neutralising with 1 M hydrochloric acid. The solvent was removed under vacuum to yield (S)-2-(acetyl-(S)-leuciny-(S)-valinyl)amino-3-phenyl propanoic acid (0.92 g, 95%). This compound (0.87 g, 2 mmol) and N-methyl piperidine (0.25 g, 2.5 mmol) was dissolved in a mixture of dry THF (50 ml) and DMF (5 ml) at room temperature under an atmosphere of nitrogen. The resultant solution was cooled to -15°C and ethyl chloroformate (0.24 g, 2.2 mmol) added. After stirring for 5 mins an ethereal solution of diazomethane (excess) was added and the mixture allowed to warm gradually to room temperature over 2 hrs. The excess diazomethane was then removed by purging the solution with a stream of N₂ for 20 mins and evaporated to dryness. The residue was redissolved in ethyl acetate (100 ml), washed with saturated sodium carbonate (2 x 50 ml), brine (1 x 50 ml), and dried over sodium sulphate. The product was purified on a silica column pretreated with triethylamine to give the diazoketone (O) (0.81 g, 92%) as a light yellow solid, mp 220-230°C (dec.). ¹H NMR (d₆ DMSO): δ 8.30 (d, *J* = 6.70 Hz, 1H, Phe-NH), 8.03 (d, *J* = 7.05 Hz, 1H, Leu- NH), 7.6 (d, *J* = 8.9 Hz, 1H, Val-NH), 7.23 (m, 5H, ArH), 6.03 (br.s, 1H, CHN₂), 4.48 (m, 1H, Phe-αCH), 4.27 (m, 1H, Leu-αCH), 4.05 (dd, *J* = 7.3, 8.9 Hz, 1H, Val-αCH), 3.02 (dd, *J* = 4.8, 16.1 Hz, 1H, Phe-βCH), 2.75 (dd, *J* = 4.8, 16.1 Hz, 1H, Phe-βCH), 1.85 (m, 1H, Val-βCH), 1.83 (s, 3H, acetyl), 1.55 (m, 1H, Leu-γCH), 1.36 (m, 1H, Leu-βCH₂), 0.86 (d, *J* = 6.73 Hz, 3H, Leu-δCH₃), 0.81 (d, *J* = 6.73 Hz, 3H, Leu-δCH₃), 0.75 (d, *J* = 4.8 Hz, 3H, Val-γCH₃), 0.72 (d, *J* = 4.8 Hz, 3H, Val-γCH₃); ISMS 444 (M + H). HRMS calcd f r C₂₃H₃₃N₃O₄ (M-N₂), 415.2583; found 415.2578.

(S)-2-(Acetyl-(S)-leuciny-(S)-valinyl)amino-1-bromo-3-phenyl butan-2-one (N)

The diaz ketone (O) (0.75 g, 1.7 mmol) was dissolved in ethyl acetate (10 ml) and treated with HBr under identical conditions used for the preparation of 5. Purification by column chromatography (silica; 50% ethyl acetate in hexane) afforded the ketobromide (X) (0.82 g, 99%) as a colorless solid: mp 184-185°C; ¹H NMR (d₆ DMSO): δ 8.5 (d, *J* = 6.8 Hz, 1H, Phe-NH), 8.0 (d, *J* = 6.6 Hz, 1H, Leu-NH), 7.7 (d, *J* = 7.0 Hz, 1H, Val-NH), 7.2 (m, 5H, ArH), 4.67 (m, 1H, Phe-αCH), 4.38 (d, *J* = 14.6 Hz, 1H, CHBr), 4.30 (d, *J* = 14.6 Hz, 1H, CHBr), 4.25 (m, 1H, Leu-αCH), 4.03 (m, 1H, Val-αCH), 3.11 (dd, *J* = 6.1, 15.9 Hz, 1H, Phe-βCH), 2.78 (dd, *J* = 6.1, 15.9 Hz, 1H, Phe-βCH), 1.8 (s, 3H, acetyl), 1.55 (m, 1H, Leu-γCH), 1.35 (m, 2H, Leu-βCH₂), 0.86 (d, *J* = 6.5 Hz, 3H, Leu-δCH₃), 0.81 (d, *J* = 6.5 Hz, 3H, Leu-δCH₃), 0.76 (d, *J* = 6.7 Hz, 3H, Val-γCH₃), 0.72 (d, *J* = 6.7 Hz, 3H, Val-γCH₃); ISMS 496/498 (M + H).

[2(R),3(S),11'(S),8'(S)]-3-[-Acetyl-(S)-leuciny-(S)-valinyl]amino-4-phenyl-1-[11'-[7',10'-dioxo-8'-(1-methylpropyl)-2'-oxa-6',9'-diazabicyclo[11.2.2] heptadeca-13',15',16'-triene]amino]butan-2-ol (M)

The macrocycle (S) (30 mg, 69 μmol) was deprotected at the N-terminus with 25% TFA in DCM according to the procedure used for the synthesis of (T). The residue was diluted with DCM (50 ml), washed with 1 M sodium hydroxide to generate the free amine, and the solvent evaporated. The residue was redissolved in THF (5ml) to which DIPEA (0.015ml, 76 μmol) and 17 (37 mg, 76 μmol) was added, the solution stirred at room temperature for 4 hs, solvent evaporated *in vacuo* and the residue redissolved in ethyl acetate (20 ml), washed with 0.5 M hydrochloric acid (5 ml), dried and the solvent removed. The ketone intermediate was not purified but reduced directly to the title compound with NaBH₄ (40 mg, 100 mmol) in MeOH (10 ml), stirred at room temperature for 30m before quenching the reaction with 1M hydrochloric acid (1 ml). Subsequent purification of the crude residue by reverse phase HPLC [gradient:

(water 0.1%TFA to 40:60 (water/ 0.1%TFA)/(water 10%/acetonitrile 90%/TFA 0.1%) over 50 mins), rt = 55.8 mins and lyophilisation gave **2a** as a colourless powder (7 mg, 15%) and the 2(S) diastereomer as the minor product. ¹H NMR (CD₃OH, 293 K) δ 8.30 (d, J = 6.05Hz, 1H, Ile-NH), 7.85 (m, 1H, 6'-NH), 7.73 (d, J = 5.8Hz, 1H, Val-NH), 7.70 (d, J = 8.9Hz, 1H, Phe-NH), 7.34 (d, J = 7.6Hz, 1H, Leu-NH), 7.23 (m, 4H, Ph), 7.17 (m, 1H, Ph), 7.11 (dd, J = 8.3, 1.9Hz, 1H, H-14'), 6.96 (dd, J = 8.4, 1.9Hz, 1H, H-17'), 6.88 (dd, J = 8.3, 2.6Hz, 1H, H-16'), 6.83 (dd, J = 8.3, 2.6Hz, 1H, H-15'), 4.38 (m, 1H, H-3'), 4.20-4.26 (m, 2H, Ile- α (CH) & H-3'), 4.13 (m, 2H, H-11' & H-2), 3.81 (m, 2H, Val- α CH & H-1), 3.54 (m, 1H, H-5'), 3.45 (m, 1H, Leu- α CH), 3.39 (dd, $J_{H11'-H12'} = 7.0$ Hz, $J_{H12'-H12} = 12.1$ Hz, 1H, H-12'), 3.28 (m, 1H, H-1), 3.08 (dd, $J_{H4-H4} = 12.7$ Hz, $J_{H4-H3} = 1$ Hz, 1H, H-4), 3.00 (dd, $J_{H4-H4} = 12.7$ Hz, $J_{H4-H3} = 6.0$ Hz, 1H, H-4), 2.79 (m, 2H, H-5' & H-12'), 2.70 (dd, $J_{H1-H1} = 14.0$ Hz, $J_{H1-H2} = 11.0$ Hz, 1H, H-1), 2.26 (m, 1H, H-4'), 2.04 (s, 3H, acetyl), 1.80-1.85 (m, 1H, Val- β CH), 1.72 (m, 1H, H-4'), 1.61-1.64 (m, 1H, Leu- γ CH), 1.53-1.59 (m, 2H, Leu- β CH & Ile- β CH), 1.43-1.49 (m, 2H, Leu- β CH & Ile- γ CH), 0.94-1.00 (m, 1H, Ile- γ CH₃), 0.93 (d, J = 6.5Hz, 3H, Leu- δ CH₃), 0.88 (d, J = 6.5Hz, 3H, Leu- δ CH₃), 0.85 (t, J = 7.4Hz, 3H, Ile- δ CH₃), 0.76 (d, J = 6.7Hz, 3H, Ile- γ CH₃), 0.74 (d, J = 6.7Hz, 3H, Val- γ CH₃), 0.59 (d, J = 6.7Hz, 3H, Val- γ CH₃). ¹³C NMR (CD₃OH): 11.69, 14.97, 18.82, 19.29, 21.79, 22.52, 23.32, 25.74, 26.47, 27.46, 30.86, 36.60, 37.25, 37.64, 39.97, 41.12, 50.72, 54.28, 54.75, 59.99, 62.17, 63.82, 68.52, 70.74, 118.57, 118.73, 127.53, 127.90, 129.46, 130.05, 130.43, 132.35, 139.36, 159.98, 167.78, 171.39, 174.19, 174.79, 175.67. ISMS: 751 (M + H). HRMS calcd for C₄₁H₆₂N₆O₇, 750.4680; found 750.4682.

[2R,3S,11'S,8'S]-3-[[[Acetyl-(S)-leuciny]- (S)-valinyl]amino]-4-phenyl-1-[7',10'-di x -8'-(-1-methylpyrpyl)-2'-xa-6',9'-diazabicyclo[11.2.2]heptadeca-13',15',16'-triene-11-yl]amin]butan-2-ol (L)

This compound was synthesised using our previously

described procedur (Abbenante *et al.*, 1995, J. Am. Chem. Soc. 117 10220-10226).

[2R,3S,12'S,9'S]-3-[[[(Acetyl-(S)-I leucynyl)-(S)-valinyl]amino]-4-phenyl-
1-[8',11'-dioxo-9'-(-1-methylpropyl)-2'-oxa-7',10'-
5 diazabicyclo[12.2.2] octadeca-14',16',17'-triene-12-yl]amino]butan-2-
ol (K)

To a stirred solution of 12-(t-butoxycarbonylamino)-8,11-
dioxo-9-(1-methylpropyl)-2-oxa-7,10-diazabicyclo-[12.2.2]octadeca-
14,16,17-triene 137. A synthesis of cycle 136 has been reported
10 (Abbenante, 1995, *et al.*, *supra*). 136 was made in three steps by
coupling 3-bromopropylamine to Boc-Ile (94%) with BOP reagent,
deprotecting (TFA), coupling with Boc-Tyr (96%) and cyclization with
base (50%). 12-(t-Butoxycarbonylamine)-8,11-dioxo-9-(1-
methylpropyl)-2-oxa-7,10-diazabicyclo[12.2.2]octadeca-14,16,17-
15 triene 137 was made similarly by coupling 4-aminobutanol to Boc-Ile,
converting the hydroxyl to bromide with CBr₄/PPh₃¹⁶, deprotecting
(TFA), coupling to Boc-Tyr adn recyclizing (~ 50% yeield overall) (30
mg, 67 μ mol) in THF (5 ml) was added DIPEA (4 eq.) and 3-(S)-
[Acetyl-(S)-leucynyl-(S)-valinyl]amino-1-bromo-4-phenyl-butan-2-one
20 (Abbenante, 1995, *et al.*, *supra*) (33 mg, 67 μ mol). The reaction
mixture was stirred for 60 mins at room temperature. The mixture
was diluted with ethyl acetate (50 ml), washed with 1M HCl dried and
the solvent removed *in vacuo*. The resultant ketoethylamine was
dissolved in methanol (10 ml), and reduced with sodium borohydride
25 (100 μ mol) by stirring the solution at -5° for 30 mins. The reaction
was quenched with acetic acid, evaported to dryness and the crude
residue purified by reverse phase HPLC [gradient: (water/0.1%TFA) to
0:100 (water/0.1%TFA):(water 10%/acetonitrile 90%/TFA 0.1%)
over 35 mins (flow rate 1.5 ml/min). Only the R- diastereoisomer was
30 isolated (6 mg, 11.7%) retention tim = 21.15 mins. ¹H nmr
(500MHz, 290K, CD₃OD): δ 8.33 (d, *J* = 5.0 Hz, 1H, Leu-NH), 8.01
(m, 1H, NH), 7.76 (d, *J* = 5.0 Hz, 1H, Val-NH), 7.65-7.73 (m, 2H,

Ile-NH, Phe-NH), 6.80-7.30 (m, 9H, ArH), 4.32 (m, 1H, H3'), 4.25 (m, 1H, Leu- α CH), 4.13-4.19 (m, 3H, Tyr- α CH, Phe- α CH, H3'), 3.80-3.87 (m, 3H, Val- α CH, Ile- α CH, H2), 3.46 (m, 1H, H6'), 3.30 (m, 2H, Phe- β CH, Tyr- β CH), 3.00-3.43 (m, 2H, H1, H1), 2.86 (m, 1H, Tyr- β CH), 2.73 (m, 1H, Phe- β CH), 2.63 (m, 1H, H6'), 2.06 (s, 3H, acetyl), 1.97 (m, 1H, H4'), 1.85 (m, 1H, Val- β CH), 1.34-1.75 (m, 8H, H4', H5', H5', Leu- γ CH, Leu- β CH₂, Ile- β CH, Ile- γ CH), 1.04 (m, 1H, Ile- γ CH), 0.95 (d, J = 6.0 Hz, 3H, Leu- δ CH₃), 0.88 (d, J = 6.0 Hz, 3H, Leu- δ CH₃), 0.87 (m, 3H, Ile- δ CH₃), 0.81 (d, J = 6.0 Hz, 3H, Ile- γ CH₃), 0.75 (d, J = 5.0 Hz, 3H, Val- γ CH₃), 0.61 (d, J = 5.0 Hz, 3H, Val- γ CH₃). ISMS 765 (M + H). HRMS calculated for C₄₂H₆₄N₆O₇, 764.4837; found 764.4840.

[2R, 3S, 11'S, 8'S]-3-[t-butoxycarbonyl]amino-4-phenyl-1-[11'-[7', 10'-dioxo-8'-(1-methylpropyl)-2'-oxa-6', 9'-diazabicyclo[11.2.2]heptadeca-13',15',16'-triene]amino]butan-2-ol (J)

11-(t-Butoxycarbonylamino)-7,10-dioxo-8-(1-methylpropyl)-2-oxa-6,9-diazabicyclo[11.2.2]-heptadeca-13,15,16-triene 136. 137 was made in 3 steps by coupling 3-bromopropylamine to Boc-Ile (94%) with BOP reagent, deprotecting (TFA), coupling with Boc-Tyr (96%), and cyclization with base (50%). 12-(t-Butoxycarbonylamino)-8,11-dioxo-9-(1-methylpropyl)-2-oxa-7,10-diazabicyclo[12.2.2]octadeca-14,16,17-triene 137 was made similarly by coupling 4-aminobutanol to Boc-Ile, converting the hydroxyl to bromide with CBr₄/PPh₃¹⁶, deprotecting (TFA), coupling to Boc-Tyr and cyclizing (~ 50% yield overall) (30 mg, 69 μ mol) was deprotected by stirring in a solution of 25% TFA in DCM at room temperature for 15 mins. The TFA was evaporated *in vacuo* and the residue dissolved in saturated NaHCO₃ solution (10 ml) and extracted with ethyl acetate (3 x 10ml). The organic phase was dried and evaporated and the residue redissolved in DMF (1 ml) and BocPhe oxide (Rich *et al.*, 1991, J. Med. Chem. 34 1222-1225) (12 mg, 46 μ mol) added. The resultant mixture was heated to 70° for 12 hrs.

The crude residue was purified by reverse phase HPLC [gradient: (water/0.1%TFA) to 50:50 (water/0.1%TFA): (water 10%/acetonitrile 90%/TFA 0.1%) over 60 mins] to give the title compound (5 mg, 18.2%), retention time = 56.20 mins, as a white powder after lyophilisation. ¹H NMR (500MHz, 290K, CD₃OD): δ 7.81 (m, 1H, NH), 6.91-7.41 (m, 10H, ArH, Ile-NH), 6.44 (d, J = 9.0 Hz, 1H, Phe-NH), 6.06 (m, 1H, Tyr-NH), 4.44 (m, 1H, H3'), 4.27 (m, 1H, H3'), 3.83 (m, 1H, H2), 3.69 (m, 2H, Tyr- α CH, Phe- α CH), 3.51 (m, 1H, Ile- α CH), 3.40 (m, 1H, H5'), 3.10-3.17 (m, 2H, Phe- β CH, Tyr- β CH), 3.03 (m, 1H, H1), 2.86-2.92 (m, 2H, H1, H5'), 2.53-2.61 (m, 2H, Phe- β CH, Tyr- β CH), 2.22 (m, 1H, H4'), 1.84 (m, 1H, H4'), 1.56 (m, 1H, Ile- β CH), 1.38 (m, 1H, Ile- γ CH), 1.25 (s, 9H, (CH₃)₃), 0.96 (m, 1H, Ile- γ CH), 0.83 (t, J = 5.0 Hz, 3H, Ile- δ CH₃), 0.73 (d, J = 5.0 Hz, 3H, Ile- γ CH₃). ISMS 597 (M+H). HRMS calculated for C₃₃H₄₈N₄O₆, 596.3574; found 596.3579.

[2S, 11'S, 8'S]-2-[t-butoxycarbonyl]amino-3-phenyl-1-[11'-[7',10'-dioxo-8'-(-1-methylpropyl)-2'-oxa-6',9'-diazabicyclo[11.2.2]heptadeca-13',15',16'-triene]amino]propane (I)

The macrocycle 136 was made in 3 steps by coupling 3-bromopropylamine to Boc-Ile (94%) with BOP reagent, deprotecting (TFA), coupling with Boc-Tyr (96%), and cyclization with base (50%). 12-(t-Butoxycarbonylamino)-8,11-dioxo-9-(1-methylpropyl)-2-oxa-7,10-diazabicyclo[12.2.2]octadeca-14,16,17-triene 137 was made similarly by coupling 4-aminobutanol to Boc-Ile, converting the hydroxyl to bromide with CBr₄/PPh₃¹⁶, deprotecting (TFA), coupling to Boc-Tyr and cyclizing (~50% yield overall) (30 mg, 69 μ mol) was deprotected as above and the resultant amine was added to a solution of BocPhe aldehyde (Fehrentz, J-A. & Castro, B., 1983, Synthesis 676-678) (17 mg, 69 μ mol), MgSO₄ (100 mg) and sodium cyanoborohydride (47 mg, 76 μ mol) in THF/1% acetic acid solution (5 ml). The mixture was stirred overnight at room temperature and quenched with 1M HCl (1 ml), evaporated in vacuo and the crude

residue purified by reverse phase HPLC [gradient: (water/ 0.1% TFA) to 0:100 (water/0.1% TFA):(water 10%/acetonitrile 90%/TFA 0.1%) over 35 mins (flow rate 1.5 ml/min), retention time = 21.24 mins. ¹H NMR (500MHz, 290K, CD3OD): δ 7.90 (m, 1H, NH), 6.82-7.37 (m, 11H, ArH, Phe-NH, Ile-NH), 6.3 (m, 1H, Tyr-NH), 4.39 (m, 1H, H3'), 4.25 (m, 1H, H3'), 4.08 (m, 2H, Tyr- α CH, Phe- α CH), 3.57 (m, 1H, H5'), 3.46 (m, 1H, Ile- α CH), 3.34 (m, 1H, Tyr- β CH), 2.68-3.07 (m, 6H, Tyr- β CH, Phe- β CH₂, H1, H1, H5'), 2.27 (m, 1H, H4'), 1.76 (m, 1H, H4'), 1.50 (m, 1H, Ile- β CH), 1.38-1.42 (m, 10H, Ile- γ CH, (CH₃)₃), 0.92 (m, 1H, Ile- γ CH), 0.86 (t, J = 6.0 Hz, 3H, Ile- δ CH₃), 0.73 (d, J = 5.0 Hz, 3H, Ile- γ CH₃). ISMS 567 (M + H). HRMS calculated for C₃₂H₄₆N₄O₅, 566.3468; found 566.3447.

[2R, 3S, 11'S, 8'S]-3-[3S-tetrahydrofuran-2-yl]amino-4-phenyl-1-[11'-[7',10'-dioxo-8'-(1-methylpropyl)-2'-oxa-6',9'-diazabicyclo[11.2.2] heptadeca-13',15',16'-triene]amino]butan-2-ol (H)

The macrocycle 136 (30 mg, 69 μ mol) was deprotected as above and the free amine reacted with 3-(S)-(3'(S)-tetrahydrofuran-2-yl)amino-1-bromo-4-phenylbutan-2-one (3-(S)-(3'(S)-tetrahydrofuran-2-yl)amino-1-bromo-4-phenylbutan-2-one was synthesised in three steps by reacting S-3-hydroxytetrahydrofuran and phenylalanine methyl ester.HCl according to the procedure of Ghosh *et al.*, 1992, Tet. Lett. 33 2781-2784 (78%), de-esterification of the resultant ester, preparation of the diazoketone (Abbenante *et al.*, 1995, *supra*) (95%) and reaction with HBr (91%) (Abbenante *et al.*, 1995, *supra*) as described for the synthesis of 5. Subsequent purification by reverse phase HPLC [gradient: (water/ 0.1% TFA) to 0:100 (water/0.1% TFA):(water 10%/acetonitrile 90%/TFA 0.1%) over 35 mins (flow rate 1.5 ml/min), gave the title compound (6 mg, 14.2%) as a white powder, retention time = 18.03 mins. The reaction gave only a very small amount of the 2(S) diastereomer which could not be isolated. ¹H NMR

(500MHz, 290K, CD3OD): δ 7.85 (m, 1H, NH), 7.37 (d, J = 5.5 Hz, 1H, II -NH), 6.83-7.32 (m, 9H, ArH), 6.44 (d, J = 9.0 Hz, 1H, Phe-NH), 5.05 (m, 1H, Furan-H), 4.40 (m, 1H, H3'), 4.26 (m, 1H, H3'), 3.64-3.89 (m, 6H, H2, Tyr- α CH, Phe- α CH, 3 Furan-H), 3.58 (m, 1H, H5'), 3.47-3.53 (m, 2H, Ile- α CH, Furan-H), 3.22 (m, 1H, Phe- β CH), 3.12 (m, 1H, H1), 3.00 (m, 1H, H1), 2.91 (m, 2H, Tyr- β CH, Phe- β CH), 2.78-2.84 (m, 2H, Tyr- β CH, H5'), 2.26 (m, 1H, H4'), 2.10 (m, 1H, Furan-H), 1.93 (m, 1H, Furan-H), 1.76 (m, 1H, H4'), 1.57 (m, 1H, Ile- β CH), 1.42 (m, 1H, Ile- γ CH), 0.99 (m, 1H, Ile- γ CH), 0.86 (t, J = 5.5 Hz, 3H, Ile- δ CH₃), 0.78 (d, J = 5.0 Hz, 3H, Ile- γ CH₃). ISMS 611 (M + H).

[2R, 3S, 11'S, 8'S]-3-[3-methylphenyl]amino-4-phenyl-1-[11'-[7',10'-dioxo-8'-(1-methylpropyl)-2'-oxa-6',9'-diazabicyclo[11.2.2]heptadeca-13',15',16'-triene]amino]butan-2-ol (G).

The macrocycle 136 (30 mg, 69 μ mol) was deprotected as above and the free amine reacted with 3-(S)-(3'-methylphenylcarbonyl)amino-1-bromo-4-phenyl-butan-2-one (3-(S)-(3'-methylphenylcarbonyl)amino-1-bromo-4-phenyl-butan-2-one was synthesized in three steps by coupling *m*-toluic acid and phenylalanine methyl ester.HCl with BOP (100%), de-esterification of the resultant ester with base, preparation of the diazoketone (Abbenante *et al.*, 1995, *supra*) (60%) and reaction with HBr (91%) (Abbenante *et al.*, 1995, *supra*) (24 mg, 69 μ mol) as described for the synthesis of 88. Purification by reverse phase HPLC [gradient: (water/0.1% TFA) to 0:100 (water/0.1% TFA):(water 10%/acetonitrile 90%/TFA 0.1%) over 35 mins (flow rate 1.5 ml/min), gave a diastereomeric mixture of 92 (7 mg, 16.5%), retention time = 20.48 mins. The NMR spectra indicated the presence of a 1:1 mixture of diastereomers by a doubling of all resonances at 290K. The spectra was consistent with the structure. Several attempts to separate diastereomers by reverse phase HPLC were unsuccessful and the diastereomeric mixture was tested for HIV-1 PR inhibition. ISMS 615 (M + H). HRMS calculated

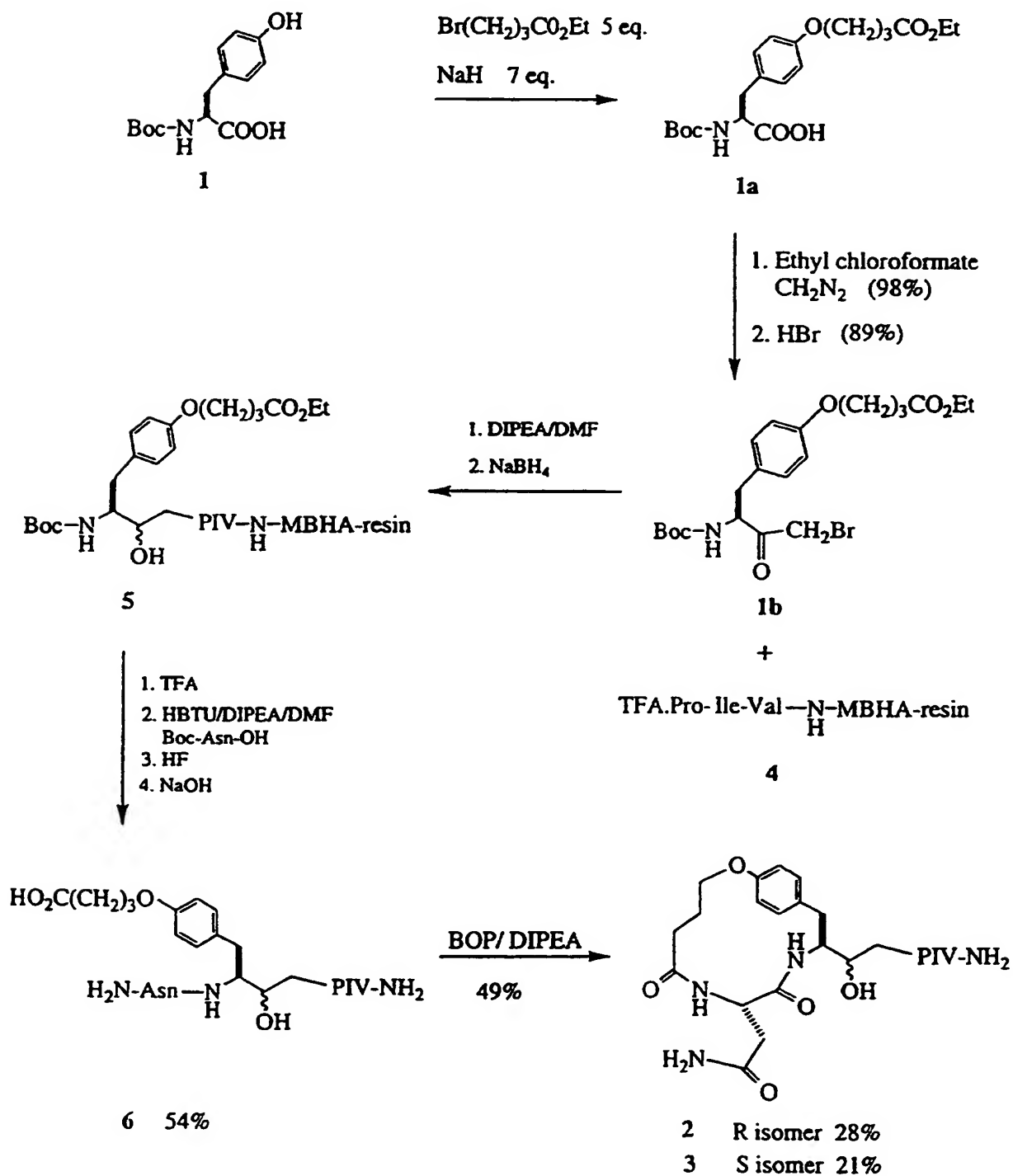
for $C_{36}H_{46}N_4O_5$, 614.3468; found 614.3467.

[2R, 3S, 11'S, 8'S]-3-[2-quin linecarbonyl]amino-4-phenyl-1-[11'-
[7', 10'-dioxo-8'-(1-methylpropyl)-2'-xa-6', 9'-
diazabicyclo[11.2.2]heptadeca-13', 15', 16'-triene]amino]butan-2-ol (E)

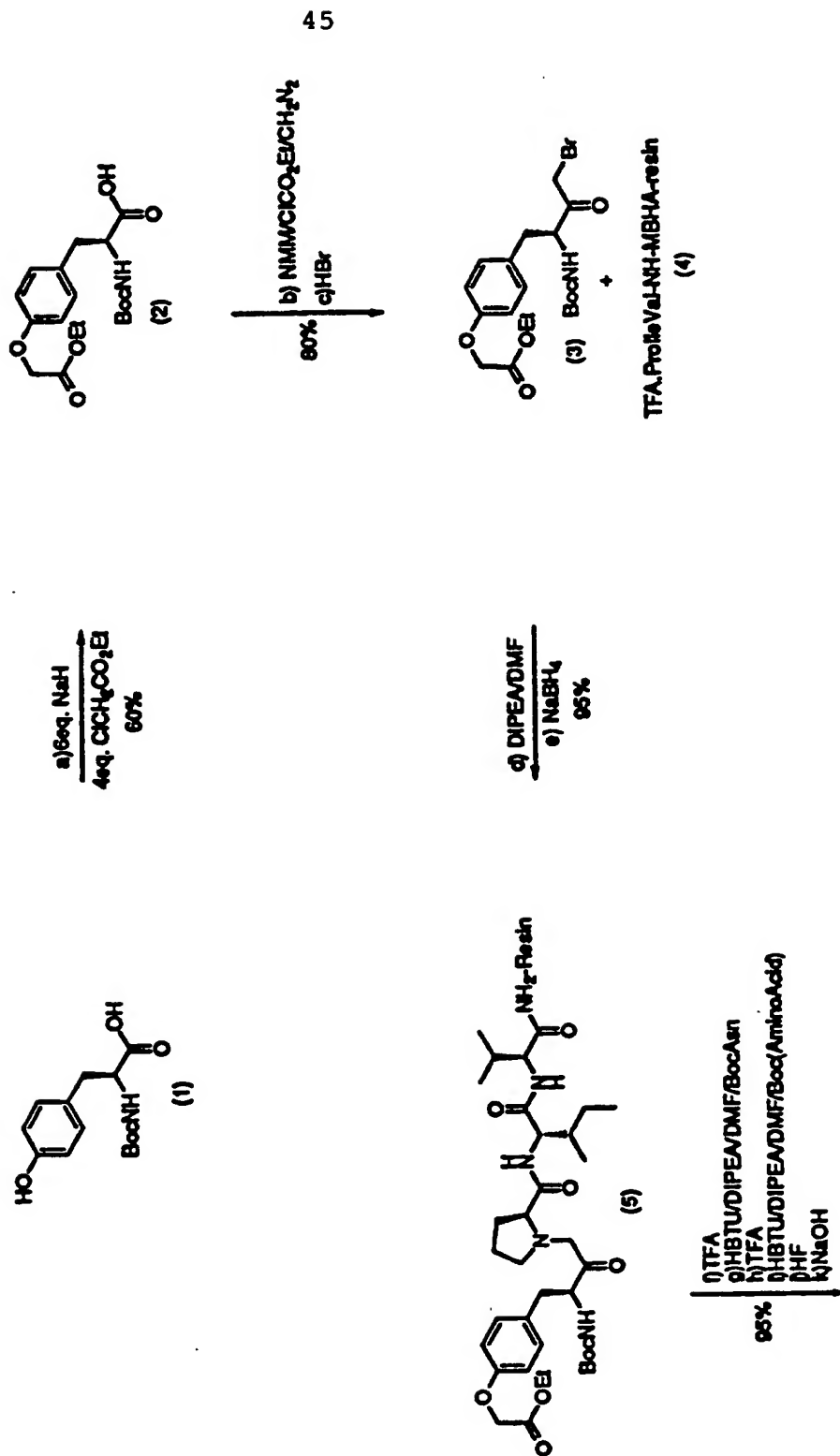
- 5 Compound 89 (3 mg, 5 μ mol) was deprotected with
25% TFA in dichloromethane (1 ml) over 15 mins and evaporated *in vacuo*. The residue was dissolved in DMF (1 ml) and to it added
quinaldyl(S)-valine (Quinaldyl(S)-valine was synthesised by the BOP
coupling of quinoline-2-carboxylic acid with Valine methyl ester.HCl
10 followed by de-esterification with NaOH) (1.6 mg, 6 μ mol), BOP (2.6
mg, 6 μ mol) and DIPEA (3 eq.) and stirred for 1 hr at room
temperature. The solvent was evaporated under reduced pressure and
the residue purified by reverse phase HPLC [gradient: (water/0.1%
TFA) to 0:100 (water/0.1% TFA):(water 10%/acetonitrile 90%/TFA
15 0.1%) over 35 mins, to give 93 (3 mg, 79%), retention time = 22.42
mins, as a white powder. 1H NMR (500MHz, 290K, CD_3OD): δ 8.77
(d, J = 7.48 Hz, 1H, Val-NH), 8.44 (d, J = 8.45 Hz, 1H, ArH), 8.25
(d, J = 8.89 Hz, 1H, Phe-NH), 8.21 (d, J = 8.45 Hz, 1H, ArH), 8.16
(d, J = 8.45 Hz, 1H, ArH), 7.99 (d, J = 8.45 Hz, 1H, ArH), 7.83-
20 7.91 (m, 2H, ArH, NH), 7.71 (m, 1H, ArH), 7.31 (d, J = 7.33Hz, 1H,
Ile-NH), 6.74-7.24 (m, 11H, ArH), 4.36 (m, 1H, H3'), 4.24 (m, 1H,
H3'), 4.11-4.17 (m, 3H, Val- α CH, Tyr- α CH, Phe- α CH), 3.78 (m, 1H,
H2), 3.56 (m, 1H, H5'), 3.47 (m, 1H, Ile- α CH), 3.29-3.33 (m, 2H,
Phe- β CH, Tyr- β CH), 3.10 (m, 1H, H1), 3.03 (m, 1H, H1), 2.79 (m,
25 1H, H5'), 2.53-2.62 (m, 2H, Phe- β CH, Tyr- β CH), 2.25 (m, 1H, H4'),
2.01 (m, 1H, Val- β CH), 1.73 (m, 1H, H4'), 1.58 (m, 1H, Ile- β CH),
1.43 (m, 1H, Ile- γ CH), 0.97 (m, 1H, Ile- γ 7.33 Hz, 3H, Ile- δ CH₃), 0.77
(d, J = 6.74 Hz, 3H, Ile- γ CH₃), 0.71 (d, J = 6.62 Hz, 3H, Val- γ CH₃).
ISMS 751 (M + H). HRMS calculated for $C_{43}H_{54}N_6O_6$, 750.4105; found
30 750.4097.

In summary, we have described a general strategy for
developing a structural and functional mimic of a tri- or tetra-peptide

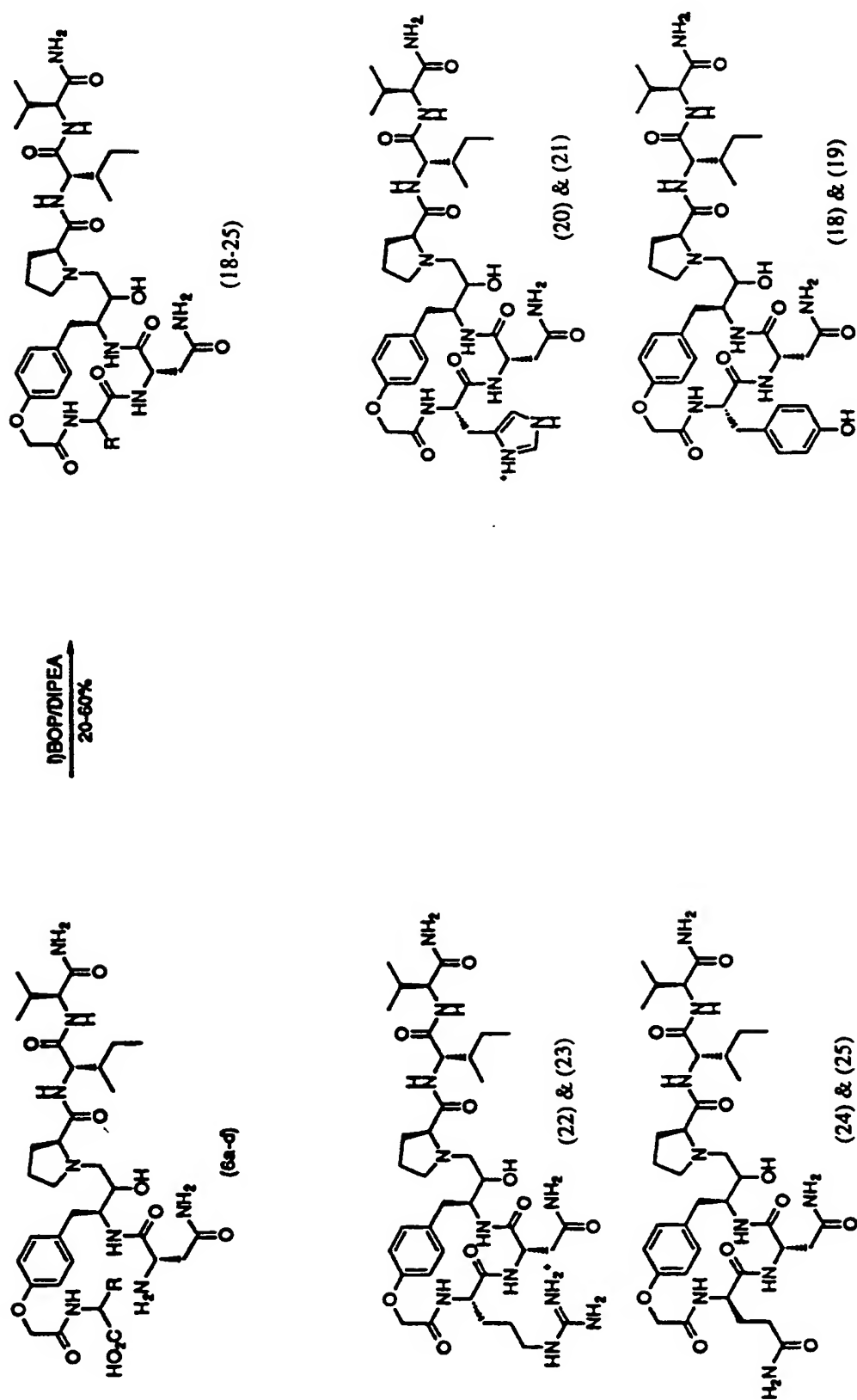
component of an inhibitor of HIV-1 protease. The ease of synthesis, variability of side chains, hydrolytic stability, ease of incorporating chiral centres, water and lipid solubility make this an attractive strategy for mimicking enzyme inhibitors.

Scheme 1 Synthesis of N-terminal cyclic inhibitors 2-17

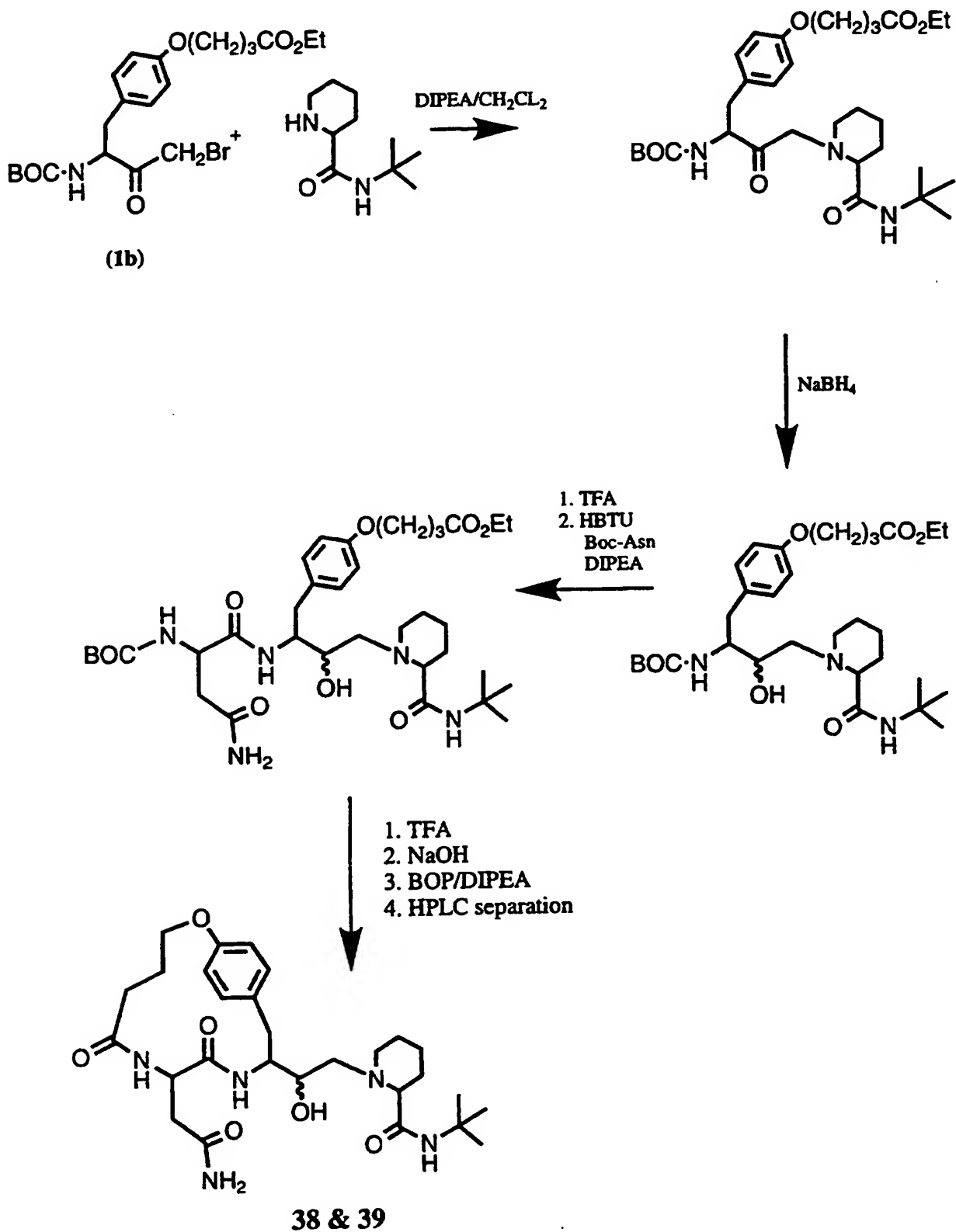
Scheme 1(Cont.) Synthesis of N-terminal cyclic Inhibitors 18-25



Scheme 1 (Cont.) Synthesis of N-terminal cyclic Inhibitors 18-25

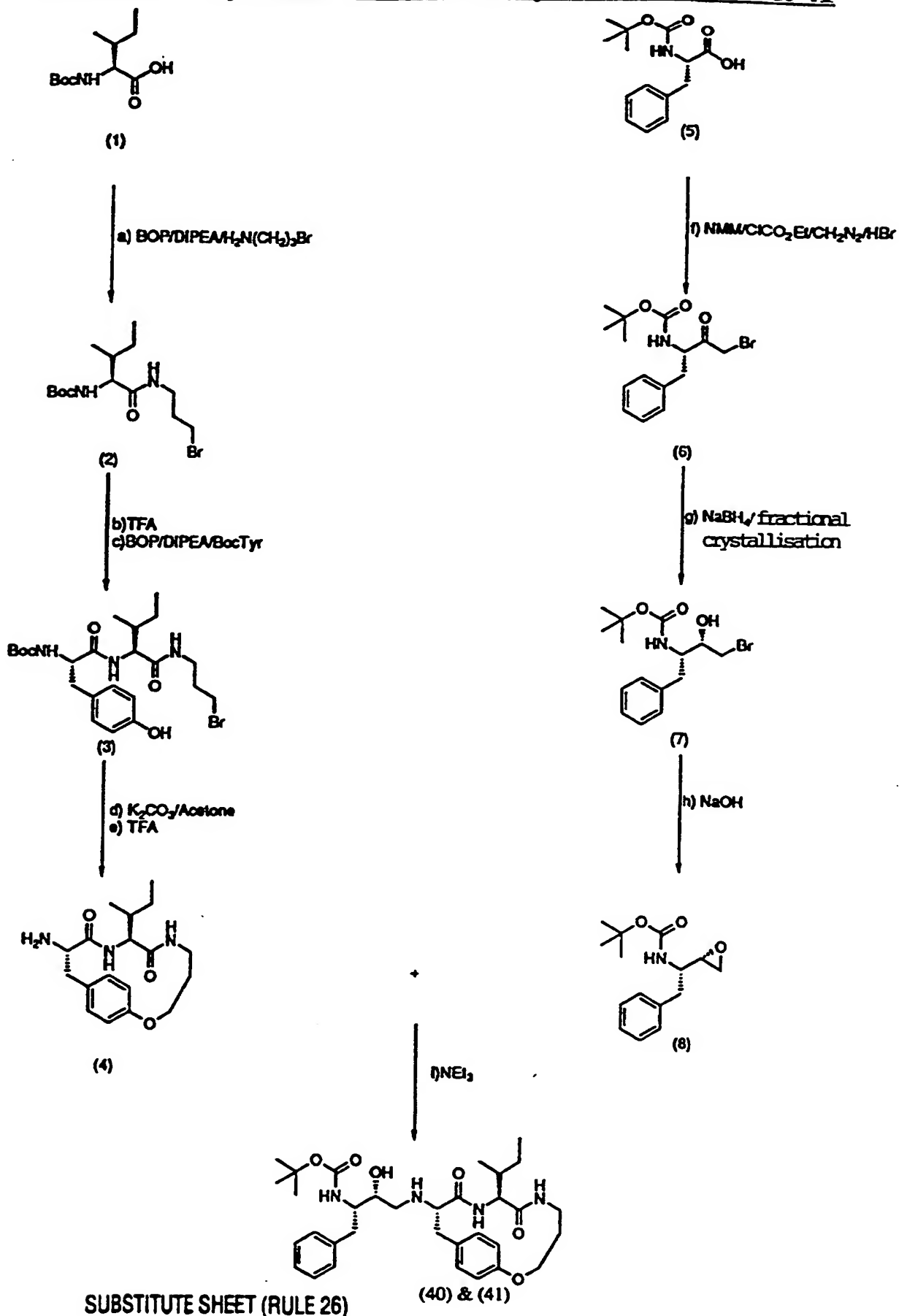


47

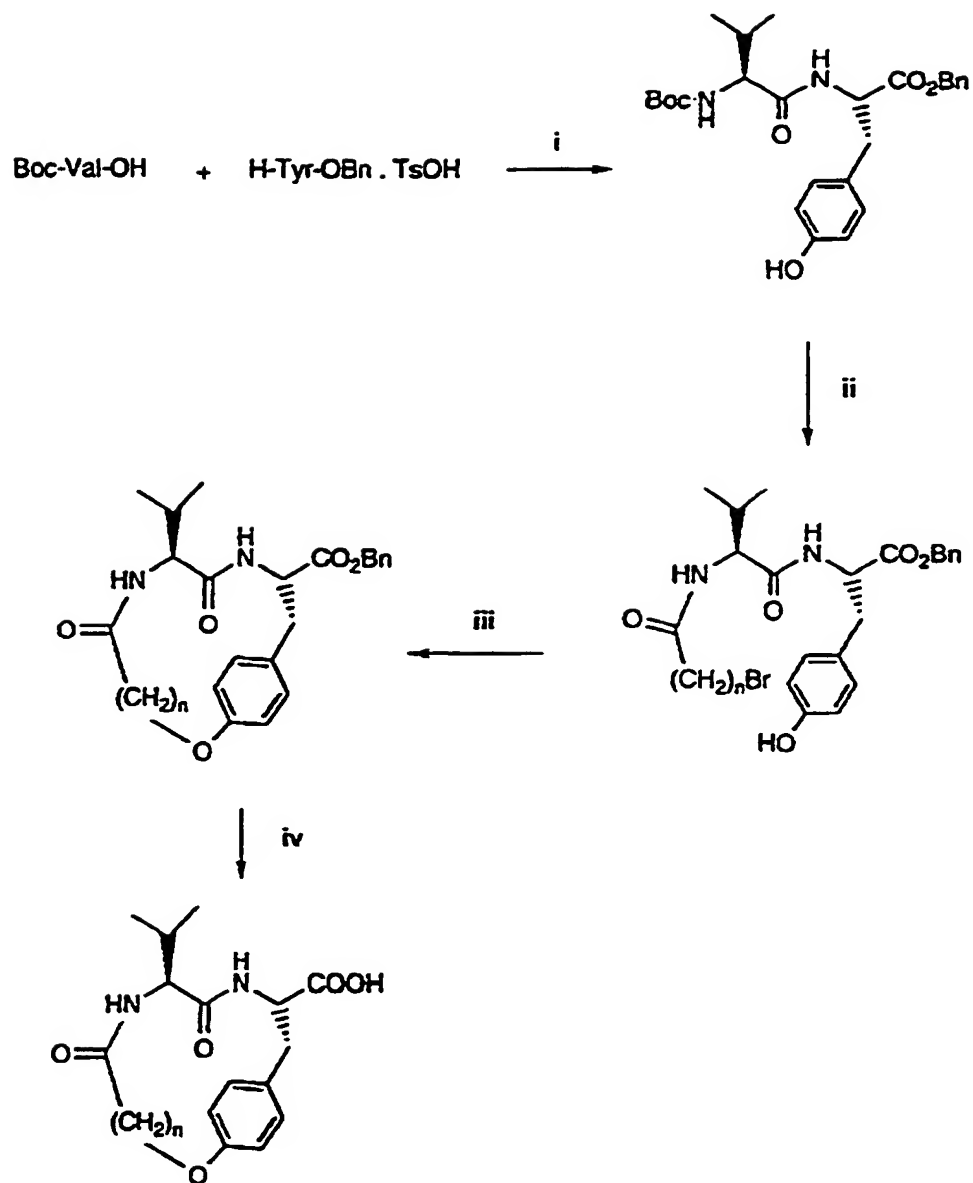
Scheme 2 Synthesis of N-terminal cyclic Inhibitors 26-39

48

Scheme 3 Synthesis of C-terminal cyclic inhibitors 40 & 41



Scheme 4

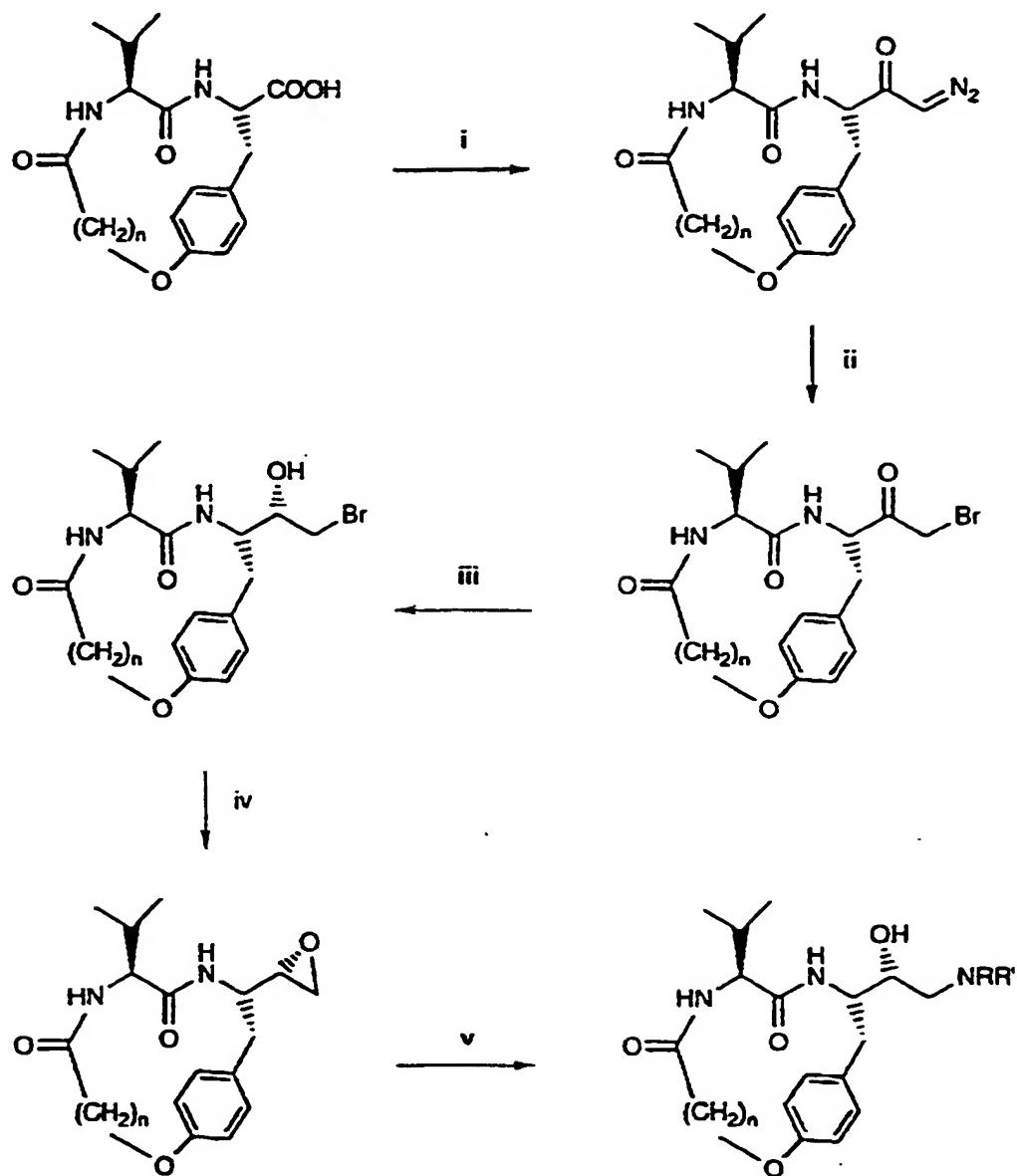


* $n=4,5$

REAGENTS: i; DCC, HOBT, Et_3N , DCM. ii; TFA. iii; $\text{Br}(\text{CH}_2)_n\text{COCl}$, KHCO_3 , THF / H_2O . iv; KO^tBu / DMF. v; H_2 / Pd, MeOH.

50

Scheme 4 (continued)



REAGENTS: i: *i*-BuOCOC_l, NMP, -15° C. ii: CH₂N₂. iii: HBr. iv: NaBH₄, EtOH, -10° C. v: NaOMe / MeOH. vi: RR'NH, DMF, 80° C.

TABLE 1

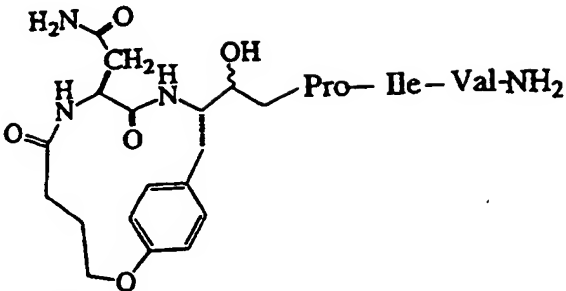
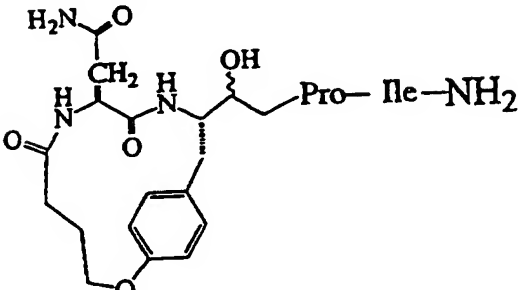
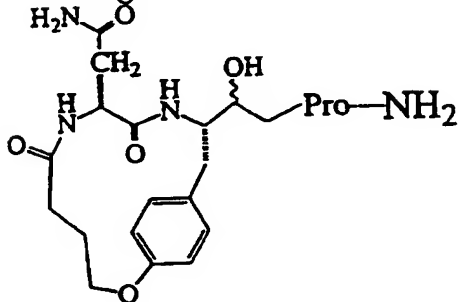
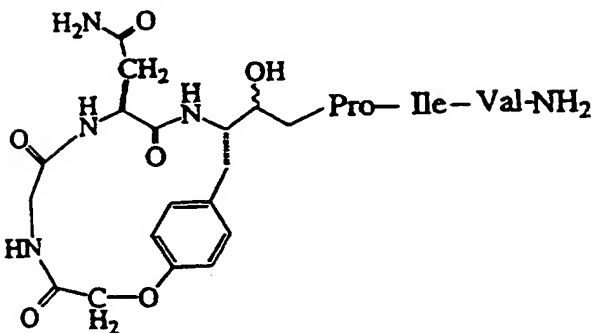
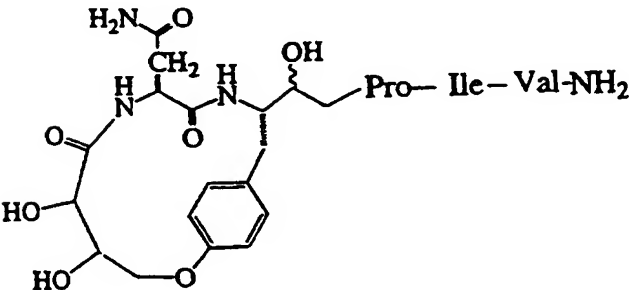
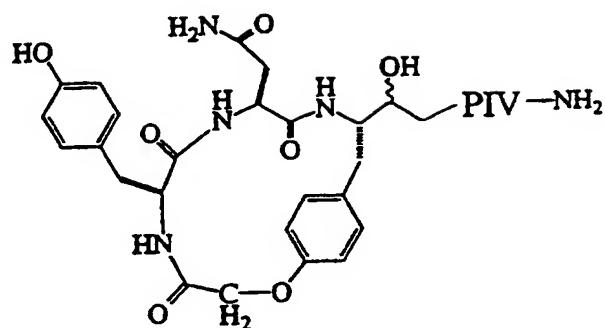
Compound		IC ₅₀ , nM
	2. R Isomer	1580
	3. S Isomer	39
	4. R Isomer	9500
	5. S Isomer	850
	6. R Isomer	55 000
	7. S Isomer	120 000
	8. R Isomer	700
	9. S Isomer	37
	10. R Isomer	600
	11. S Isomer	49

TABLE 3

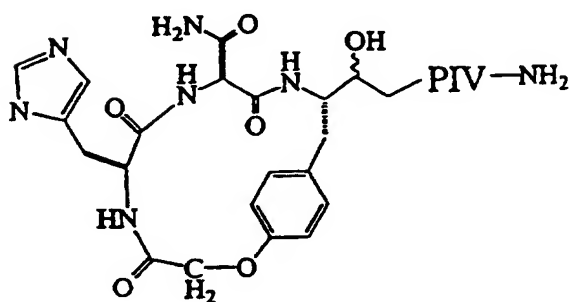
53



Compound	IC ₅₀ , nM
----------	-----------------------

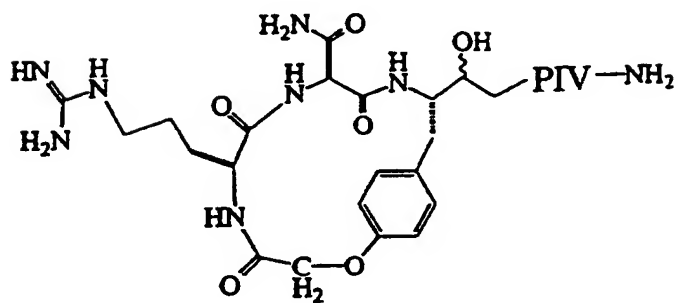
18. S Isomer	38
--------------	----

19. R Isomer	165
--------------	-----



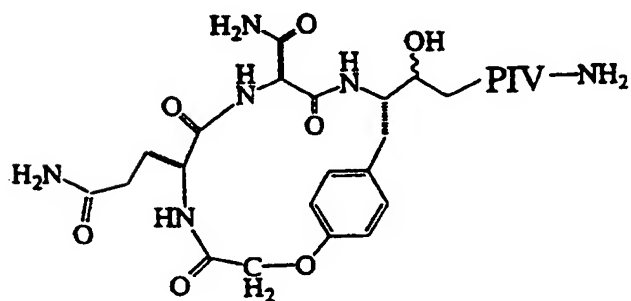
20. S Isomer	26
--------------	----

21. R Isomer	339
--------------	-----



22. S Isomer	42
--------------	----

23. R Isomer	49
--------------	----

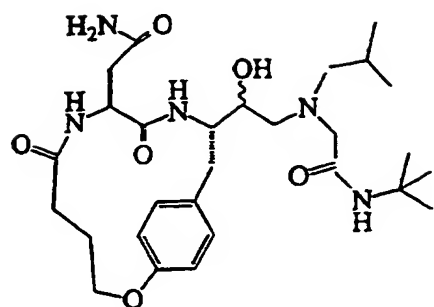


24. R Isomer	40 rac
--------------	--------

25. S Isomer	
--------------	--

TABLE 4

54



Compound

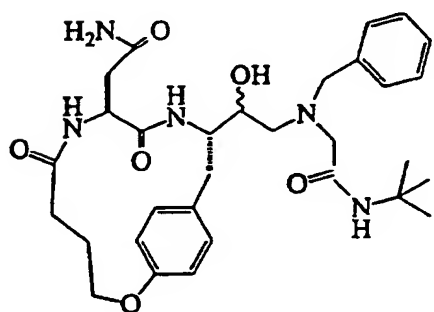
IC₅₀, nM

26. R isomer

1700

27. S isomer

1900

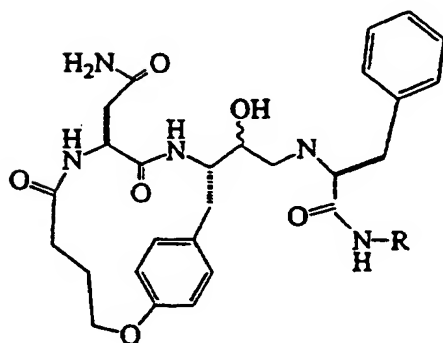


28. R isomer

1500

29. S isomer

46000



R = tBu

30. R isomer

rac 70000

31. S isomer

R = iPr

32. R isomer

rac 42000

33. S isomer

R = iBu

34. R isomer

rac 5200

35. S isomer

TABLE 5

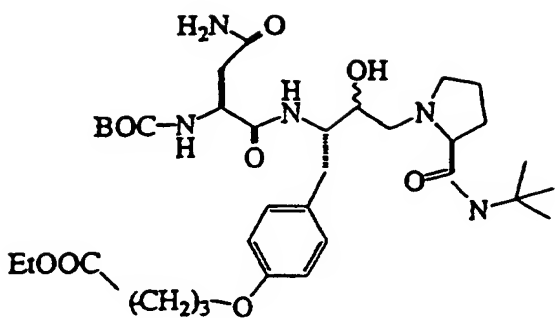
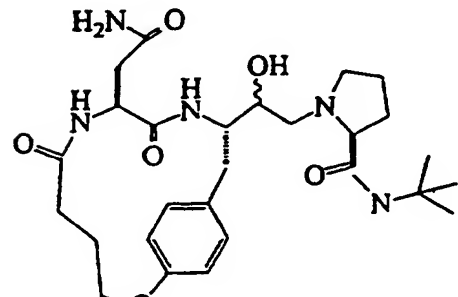
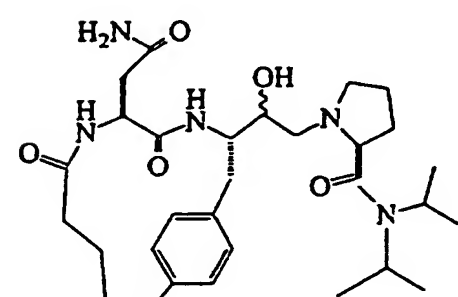
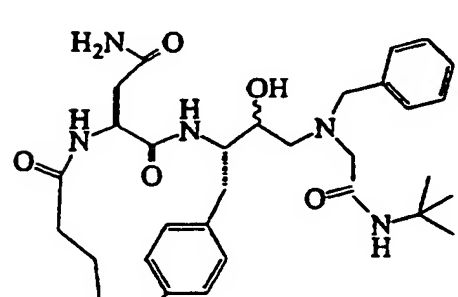
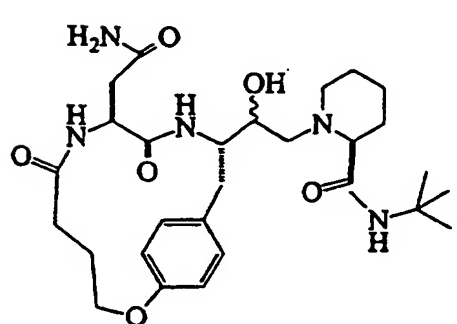
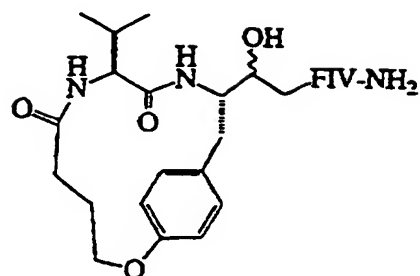
	Compound	IC ₅₀ , nM
	36. R Isomer	7050
	37. S Isomer	22000
	38. R Isomer	194
	39. S Isomer	490
	40. R Isomer	106000
	41. S Isomer	290000
	42. R Isomer	1500
	43. S Isomer	46000
	44. R Isomer	16
	45. S Isomer	5600

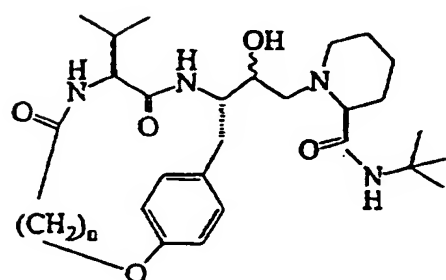
TABLE 6

56

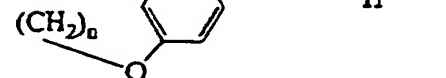
Compound IC_{50} , nM

46. R Isomer 49

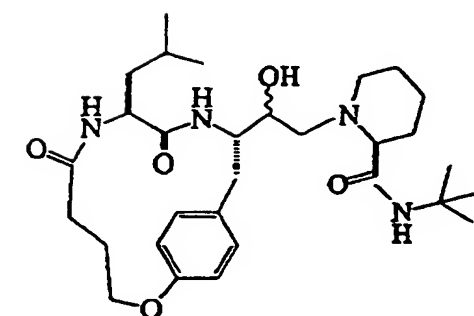
47. S Isomer

n=3
48. R Isomer 40

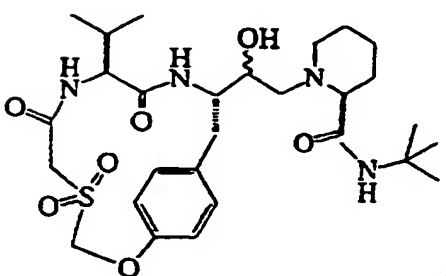
49. S Isomer

n=4
50. R Isomer 30

51. S Isomer

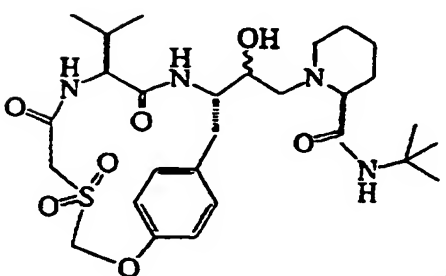
n=5
52. R Isomer 85

53. S Isomer



54. R Isomer

55. S Isomer

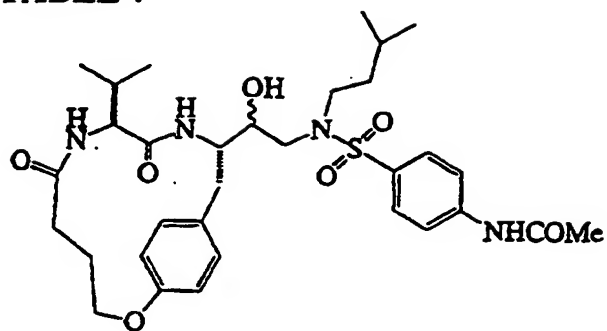


56. R Isomer

57. S Isomer

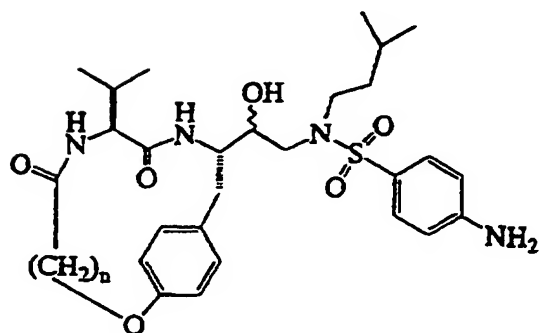
TABLE 7

57

Compound IC_{50} , nM

58. R Isomer 13

59. S Isomer



n = 3

60. R Isomer 2

61. S Isomer

n = 4

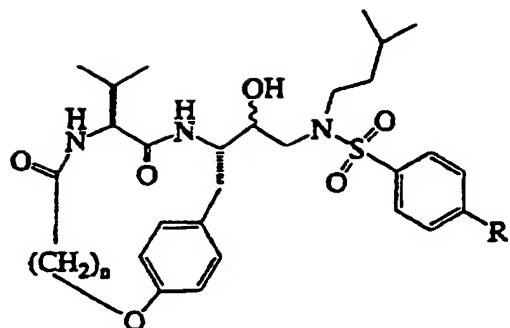
62. R Isomer 1

63. S Isomer

n = 5

64. R Isomer 2

65. S Isomer



n = 3, R = H

66. R Isomer 1.5

67. S Isomer

n = 4, R = H

68. R Isomer 0.5

69. S Isomer

n = 5, R = H

70. R Isomer

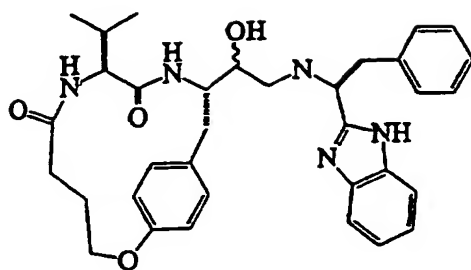
71. S Isomer

n = 4, R = CH₃

72. R Isomer

73. S Isomer

TABLE 8

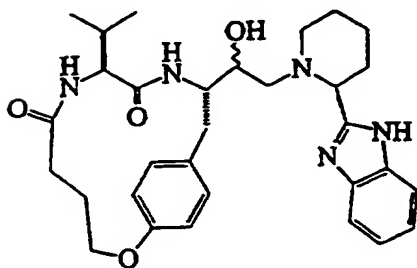


Compound

IC₅₀, nM

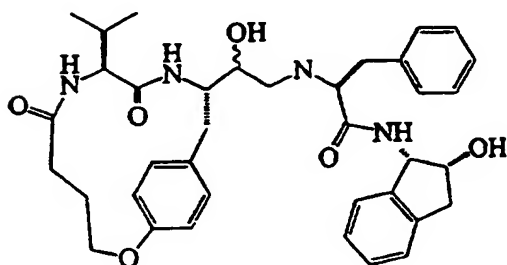
74. R Isomer

75. S Isomer



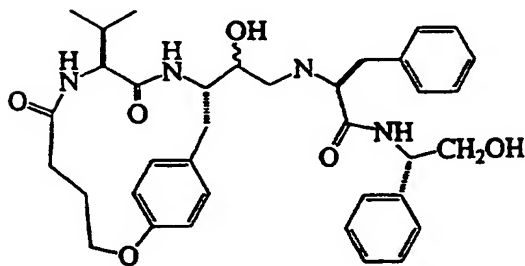
76. R Isomer

77. S Isomer



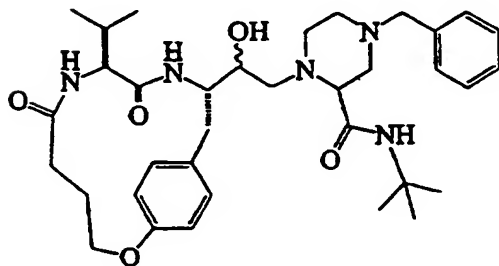
78. R Isomer

79. S Isomer



80. R Isomer

81. S Isomer

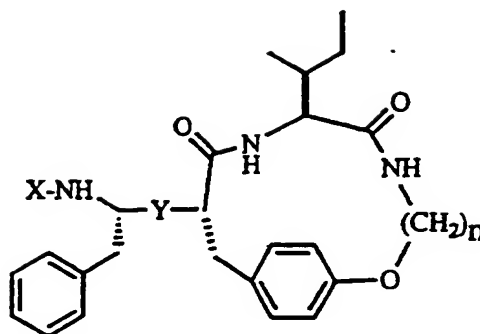


82. R Isomer

83. S Isomer

59

TABLE 9



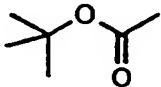
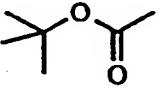
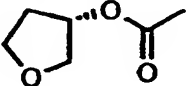
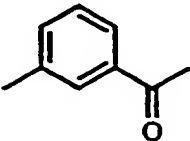
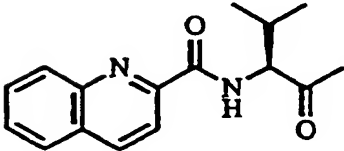
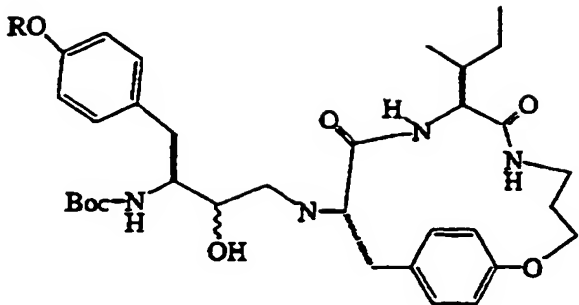
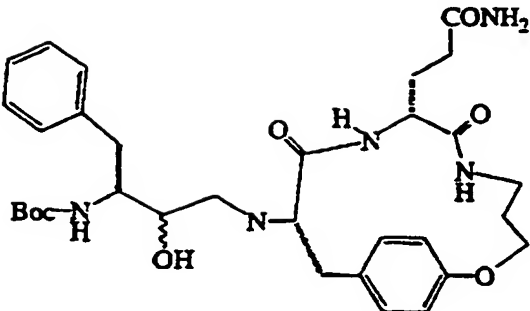
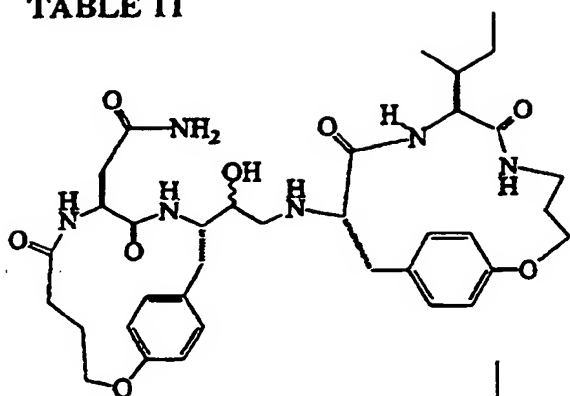
Compound	X	Y	n	IC ₅₀ , nM ^a	
84	Ac-SLNFPIV-NH ₂ ^b	-	-	R	22
				S	1
85	Ac-LNFPIV-NH ₂	-	-	R	60
				S	9
86	Ac-LVFFIV-NH ₂ ^c	-	-	R	5
				S	5
87	Ac-Leu-Val-	-CH(OH)CH ₂ NH	3	R	2
				S	2
88	Ac-Leu-Val-	-CH(OH)CH ₂ NH	4	R	5
89		-CH(OH)CH ₂ NH	3	R	15
90		-CH ₂ NH	3	R	150
91		-CH(OH)CH ₂ NH	3	R	15
92		-CH(OH)CH ₂ NH	3	R+S	50
93		-CH(OH)CH ₂ NH	3	R	1
94	DM323	-	-	-	1

TABLE 10

		Compound	IC ₅₀ , nM
	95.	R = OH R isomer	
	96.	S isomer	
	97.	R = CH ₂ CO ₂ H R isomer	
	98.	S isomer	
	99.	R = CH ₂ CO ₂ H R isomer	25
	100.	S isomer	
	101.	R = CH ₂ CO ₂ Me R isomer	15
	102.	S isomer	
	103.	R = CH ₂ CO ₂ Bu R isomer	5
	104.	S isomer	
105.	R = CH ₂ CO ₂ Hex R isomer	2	
106.	S isomer		
	107.	R isomer	250
	108.	S isomer	

61

TABLE 11

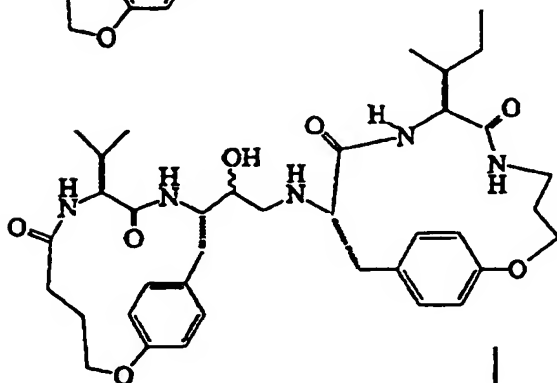


Compound

IC₅₀, nM

109. R isomer

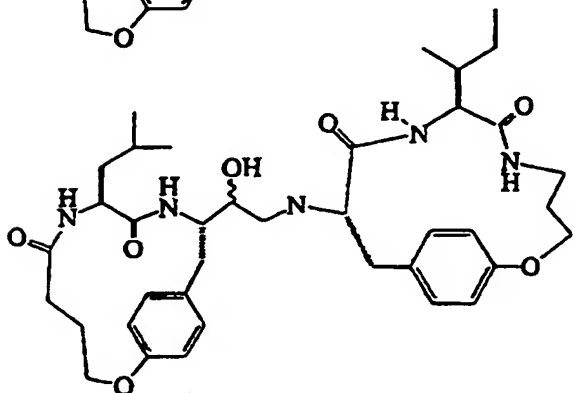
110. S isomer



111. R isomer

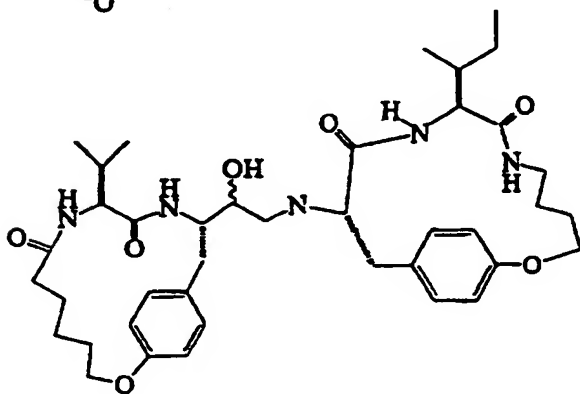
8

112. S isomer



113. R isomer

114. S isomer



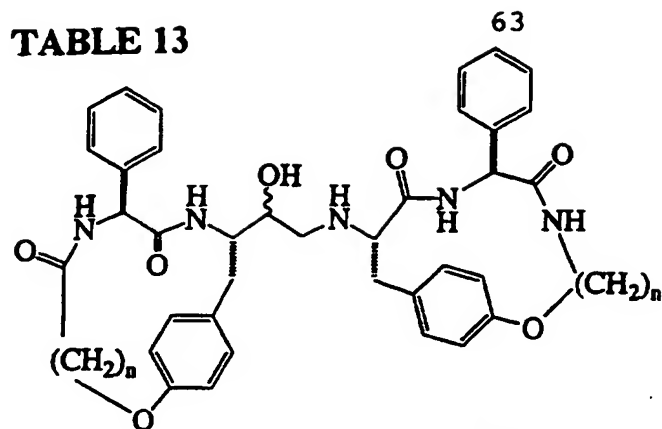
115. R isomer

116. S isomer

TABLE 12

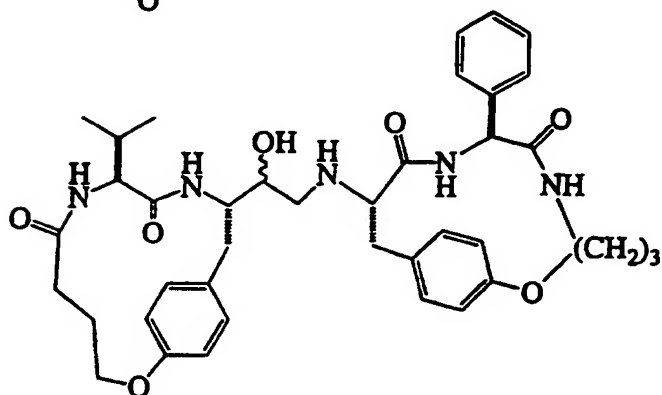
	Compound	IC ₅₀ , nM
	117. R isomer	
	118. S isomer	
	119. R isomer	
	120. S isomer	
	121. R isomer	
	122. S isomer	
	123. R isomer	
	124. S isomer	
	125. R isomer	
	126. S isomer	

TABLE 13

Compound IC_{50}, nM

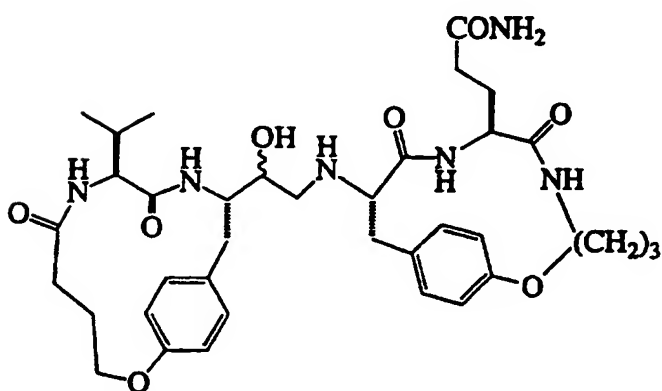
127. R isomer

128. S isomer



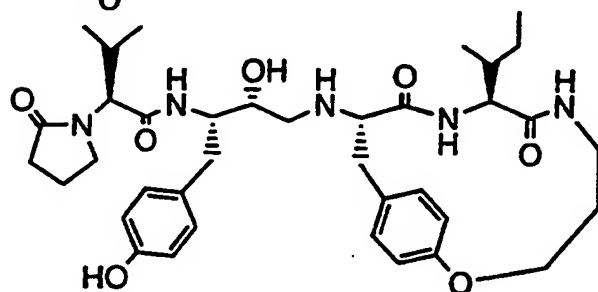
129. R isomer

130. S isomer

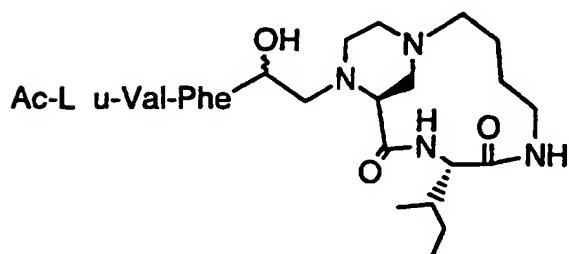


131. R isomer

132. S isomer



133.



134.

TABLE 14 Data collection and *r* finement statistics for HIVPR and HIVKI inhibitor c mplexes

Inhibitor	3 l.h.s.-PIV	44 l.h.s.-tBu	89 r.h.s.-tBu	133 r.h.s.-5c-Tyr
Protease	HIVPR Aba 67/95	HIVKI Aba 67/95	HIVKI Aba 67/95	HIVKI Aba 67/95
Resolution limit	2.0 Å	1.75 Å	1.75 Å	1.85 Å
R_{sym}	0.080	0.067	0.068	0.063
% complete-ness of data	97.1 %	82.5 %	85.9%	80.5%
Unit cell	51.3 Å	51.4 Å	51.4 Å	51.6 Å
parameters	58.8 Å	59.3 Å	58.7 Å	59.1 Å
(a, b, c)	62.3 Å	61.9 Å	62.0 Å	62.4 Å
Number of observations ($I/\text{sigI} > 1.0$)	59,803	52,113	88,133	39,617
Number of unique reflections	11,924	16,268	16,528	13,620
	8-2.0 Å	8-1.75 Å	8-1.75 Å	8-1.85 Å
Refinement Statistics				
Final R-factor	0.163	0.177	0.179	0.172
(R-free)	(0.237)	(0.239)	(0.244)	(0.239)
RMS bl	0.009 Å	0.010 Å	0.010 Å	0.009 Å
RMS ba	1.59°	1.55°	1.66°	1.65°
RMS dihe	26.9°	26.1°	26.1°	26.4°
RMS impr	1.42°	1.29°	1.40°	1.40°
Number of waters	95	107	115	114
Number of sulphates	3	3	3	3
Sidechains modelled as Ala	Lys 41, 43, 45, 70, 141, 143	Lys 41, 43, 143	Lys 41, 43, 141, 143	Asn37, Lys 41, 43, 45, 55, 70, 141

TABLE 14 continu d

Residues for which two sidechain conforma- tions were modelled	-	Arg8, Glu34, Arg57, Gln61, Glu65, Gln118, Glu135, Met146, Lys155	Glu34, Glu35, Ile50, Gly51, Arg57, Met146, Ile150, Gly151	Glu35, Ile50, Gly51, Gln61, Val82, Ile150, Gly151, Val182
--	---	--	--	--

LEGENDS**FIG. 1**

Molecular model of cyclic inhibitor 3 (dark outline) superimposed on the protease bound conformation of JG-365 (light outline)

5 FIG. 2

Overlay of the crystal structure of cyclic inhibitor 3 (bound to HIVPR) superimposed on the crystal structure of JG-365 (bound to HIVPR)

FIG. 3

10 Mass spectral analysis of cyclic inhibitor (12) after incubation (37°C, 1h, 5 units each enzyme) with human cathepsin D, pepsin 3a or gastrixin

FIG. 4

15 Mass spectral analysis of cyclic inhibitor (10) after incubation (37°C, 1h, 5 units each enzyme) with human cathepsin D, pepsin 3a or gastrixin

FIG. 5

Mass spectral analysis of cyclic inhibitor (11) after incubation (37°C, 1h, 5 units each enzyme) with human cathepsin D, pepsin 3a or gastrixin

20 FIG. 6

Mass spectral analysis of cyclic inhibitor (8) after incubation (37°C, 1h, 5 units each enzyme) with human cathepsin D, pepsin 3a or gastrixin

FIG. 7

25 Mass spectral analysis of cyclic inhibitor (9) after incubation (37°C, 1h, 5 units each enzyme) with human cathepsin D, pepsin 3a or gastrixin

FIG. 8

30 Mass spectral analysis of cyclic inhibitor (3) after incubation (37°C, 1h, 5 units each enzyme) with human cathepsin D, pepsin 3a or gastrixin

FIG. 9

Mass spectral analysis of cyclic inhibitor (2) after incubation (37°C, 1h, 5 units each enzyme) with human cathepsin D, pepsin 3a or gastrin

FIG. 11

- 5 Overlay of receptor bound conformations of 3 (modelled structure) [199] on JG-365 (x-ray structure) [197]

FIG. 12

Overlay of receptor bound conformations of 87 (modelled structure) [203] on JG-365 (x-ray structure) [197]

- 10 **FIG. 13**

Overlay of receptor bound conformations of 134 (modelled structure) [203] on JG-365 (x-ray structure) [197]

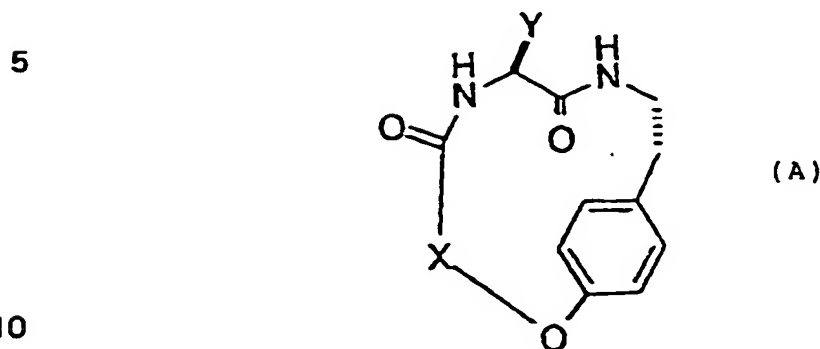
FIG. 14

- 15 Overlay of receptor bound conformations of 111 (modelled structure) [203] on JG-365 (x-ray structure) [197]

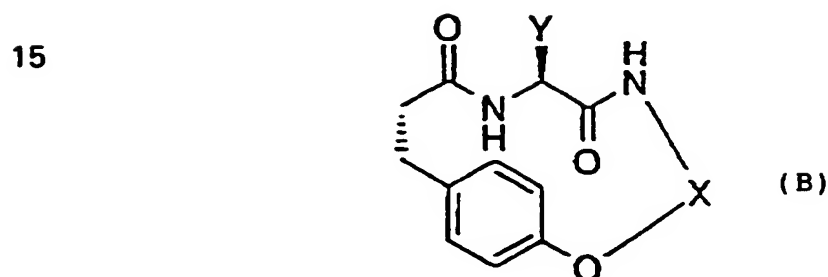
68

CLAIMS

1. A HIV-1 protease inhibitor which includes an N-terminal cycle



or a C-terminal cycle



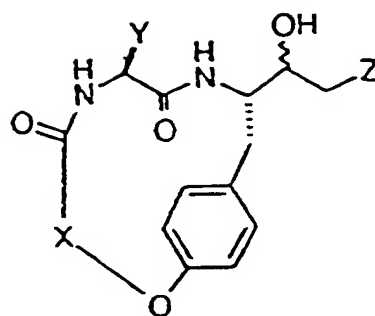
or both cycles (A) and (B)

wherein Y is selected from side chains of Asn or Ile or Val or Glu and alkyl of 1-6 carbon atoms inclusive of linear or branched chains as well as cycloalkyl; and

- 25 X is selected from $(CH_2)_n$ where $n = 3-6$, $-CH(OH)-CH(OH)-CH_2-$, $CH(CO_2H)-CH_2-CH_2$, $CH_2CONHCHR$ where R = D or L amino acids and alkyl of 1-6 carbon atoms inclusive of linear or branched chains.

2. A HIV-1 protease inhibitor as claimed in Claim 1 which
30 includes compounds of structure

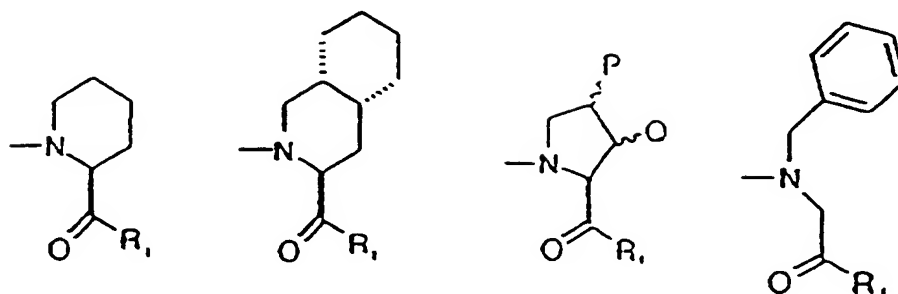
5 N-Terminus Cyclic Inhibitors (i)



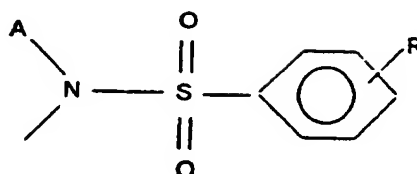
wherein Z is selected from

10

15



20

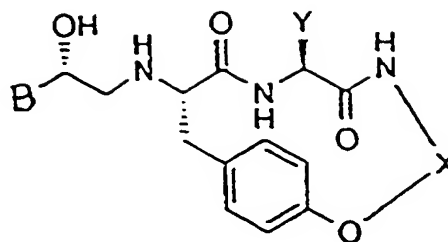


- wherein
- P = H, alkyl, aryl, Oalkyl, Nalkyl
- Q = H, alkyl, aryl, Oalkyl, Nalkyl
- 25 R₁ = NHtBu, OtBu, NHiBu, NHiPr, NHnBu, NHnPr, NHalkyl, Oalkyl; or
- A = alkyl of 1-6 carbon atoms inclusive of linear or branched structures as well as cycloalkyl; and
- 30 R = is selected from amino, O-alkyl or N-alkyl.

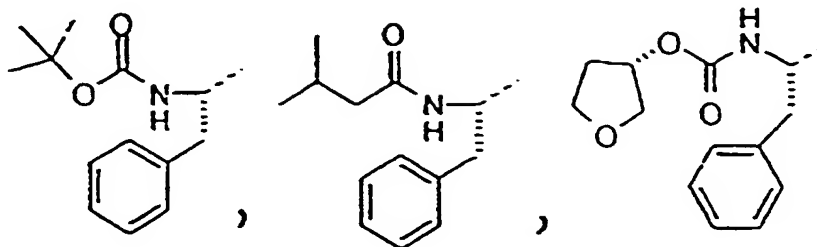
3. A HIV-1 proteas inhibitor as claimed in Claim 1 having the structure

70

C-Terminus Cyclic Inhibitors (ii)



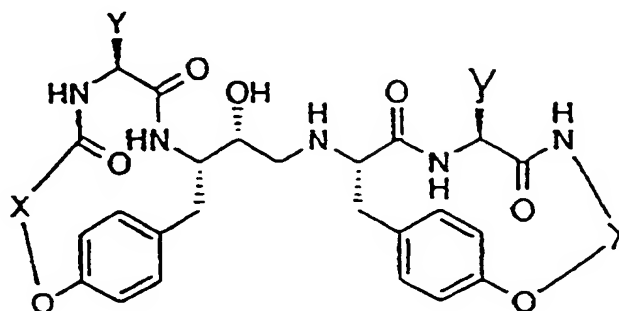
wherein B is selected from



or quinoline - Val-Phe-.

4. A HIV-1 protease inhibitor as claimed in Claim 1 having the structure

Bicyclic Inhibitors (iii)



5. Compounds 1-137 herein excluding compound 94.

1/12

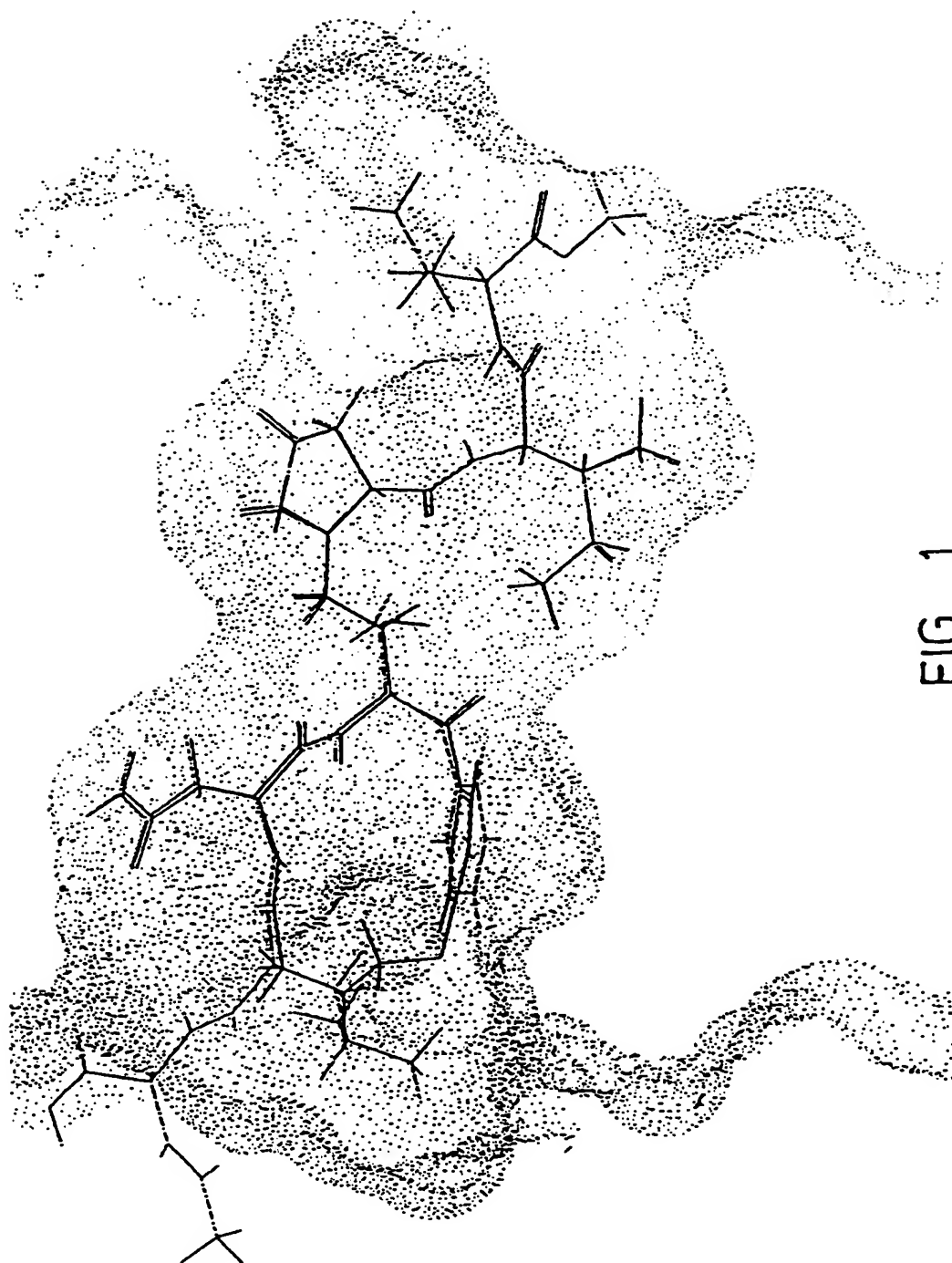


FIG. 1

2/12

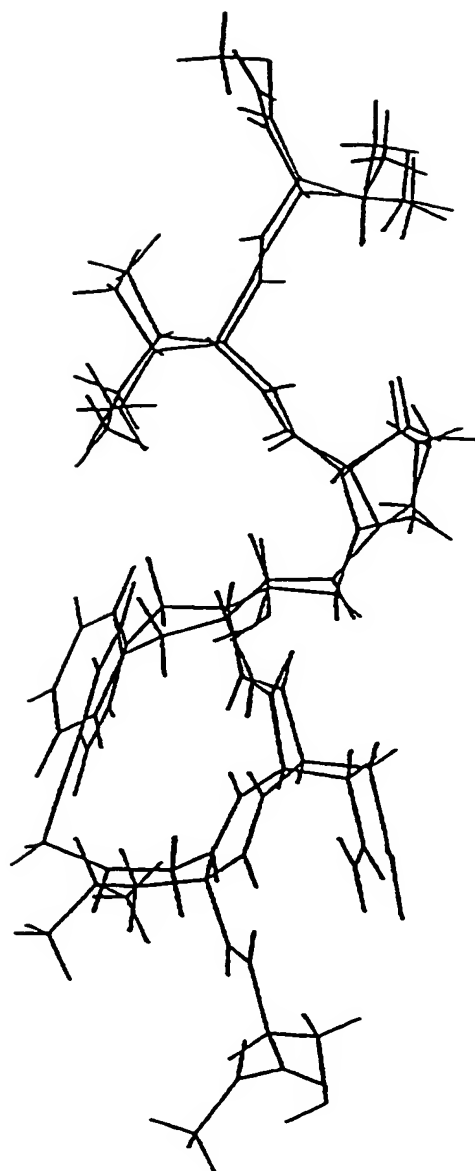
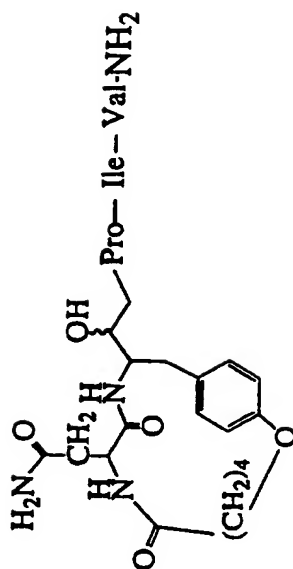


FIG. 2

3/12



18/11/1993 15:34

File: B709Y93

240A93 PH3 B1=69 B2=150 R1/R2=14/14

B709Y93'1 (3.571) Sm(DF:2x2): (MS), ES+

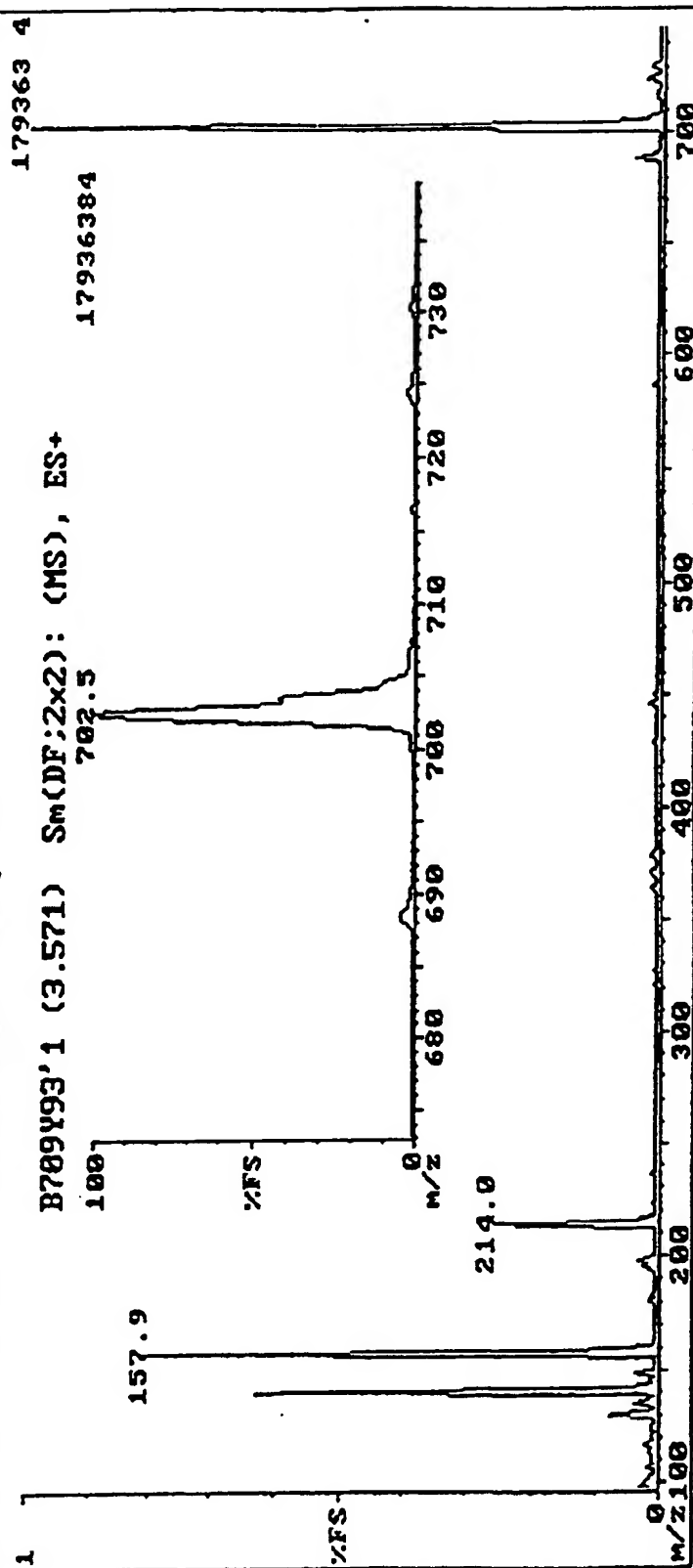


FIG. 3

4 / 12

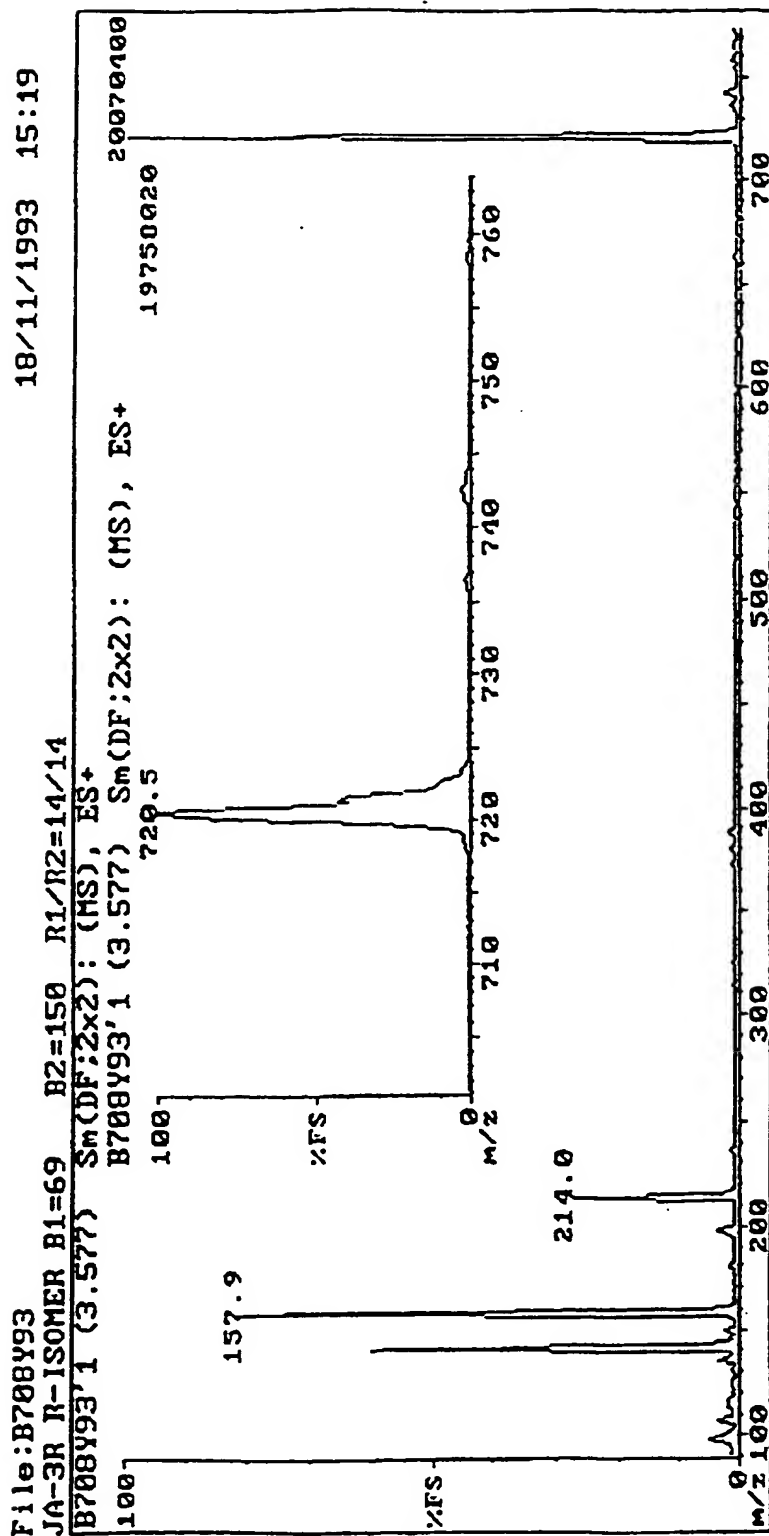
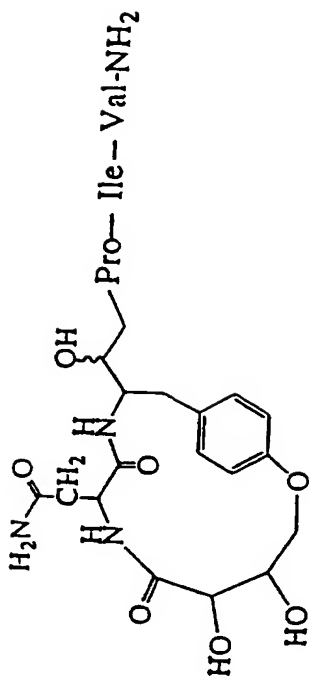
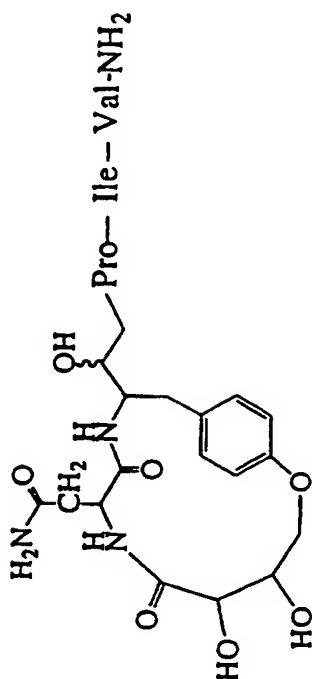


FIG. 4



File:B702493

18/11/1993 13:28

JA-3S S-ISOMER B1=69 B2=150 R1/R2=14/14

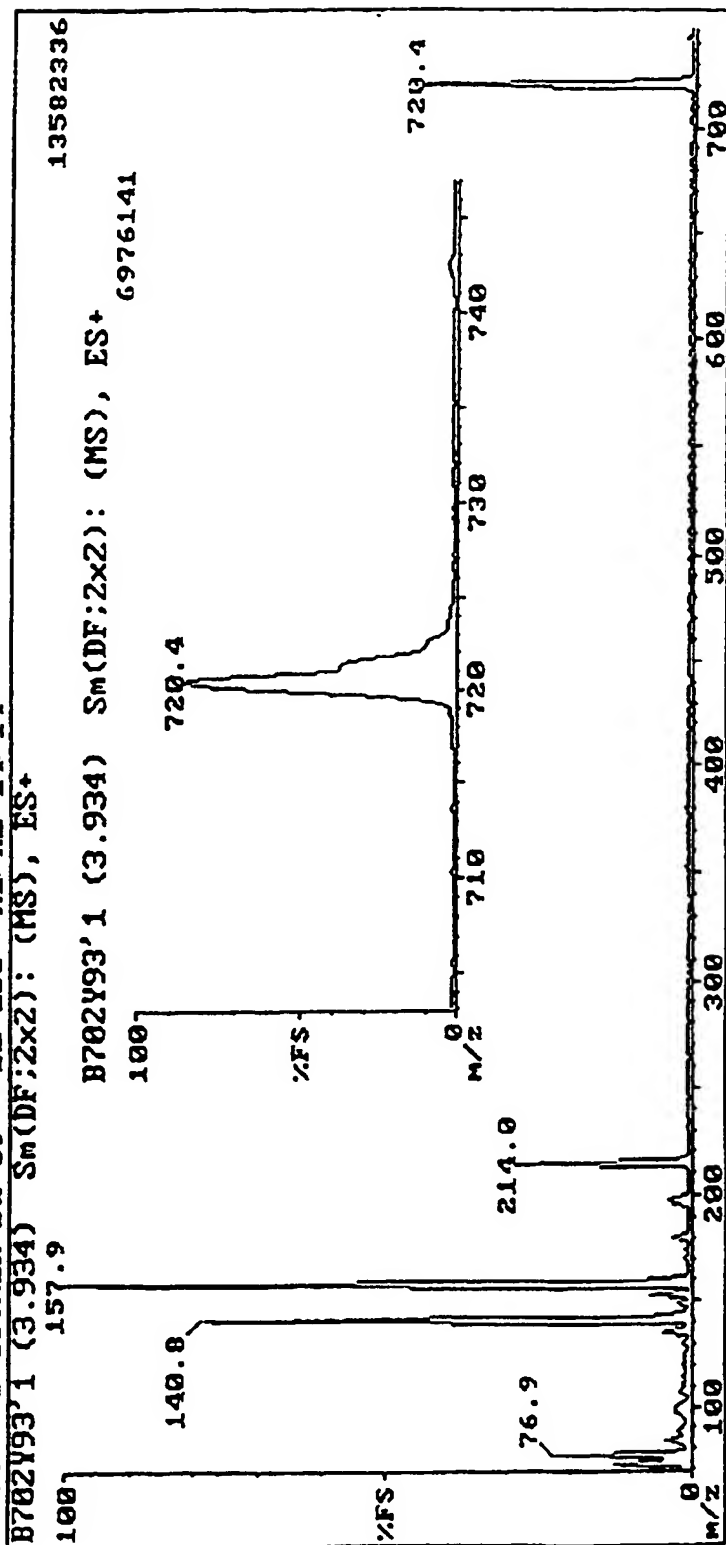
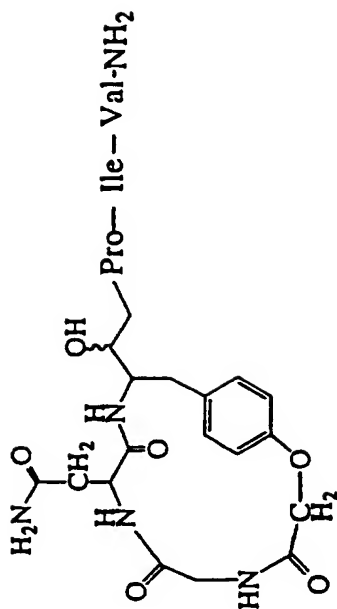


FIG. 5

6/12



18/11/1993 15:06

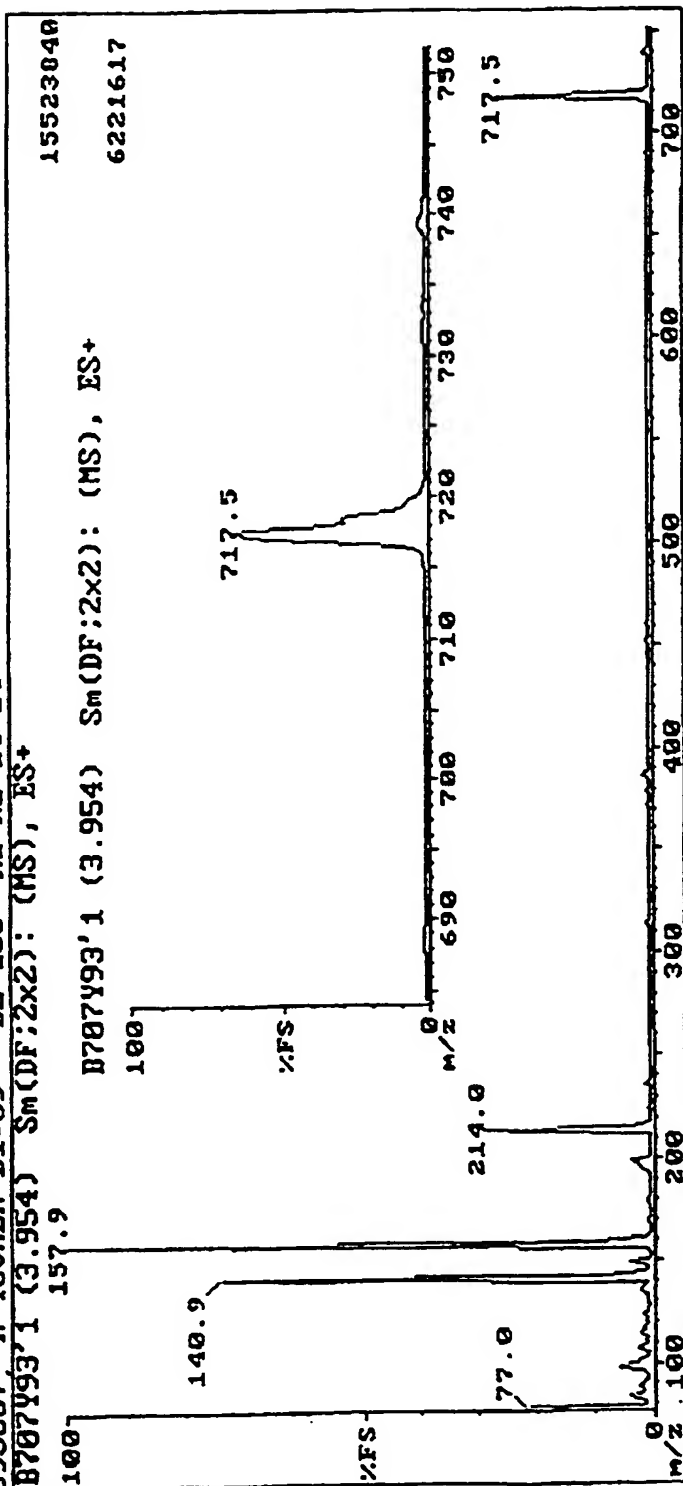
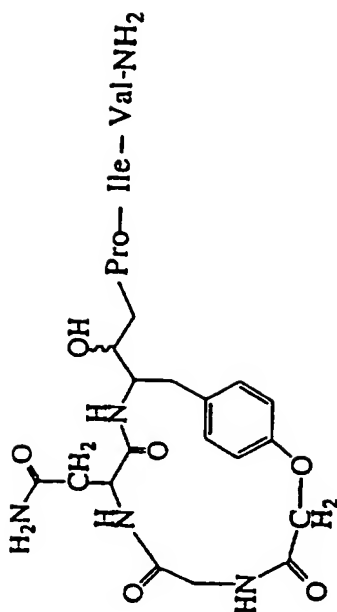
File: B707Y93
S93067 R-ISOMER B1=69 B2=150 R1/R2=14/14

FIG. 6

7/12



File:D701493
S930066 S-ISOMER B1=69 B2=150 R1/R2=14/14
18/11/1993 13:15

18/11/1993 13:15

B2=150 R1/R2=14/14

(2): (MS), ES+

$\chi^2_{(93)} = 1$ (3.771) $S_M(DF; 2 \times 2)$: (MS), ES+

3633821

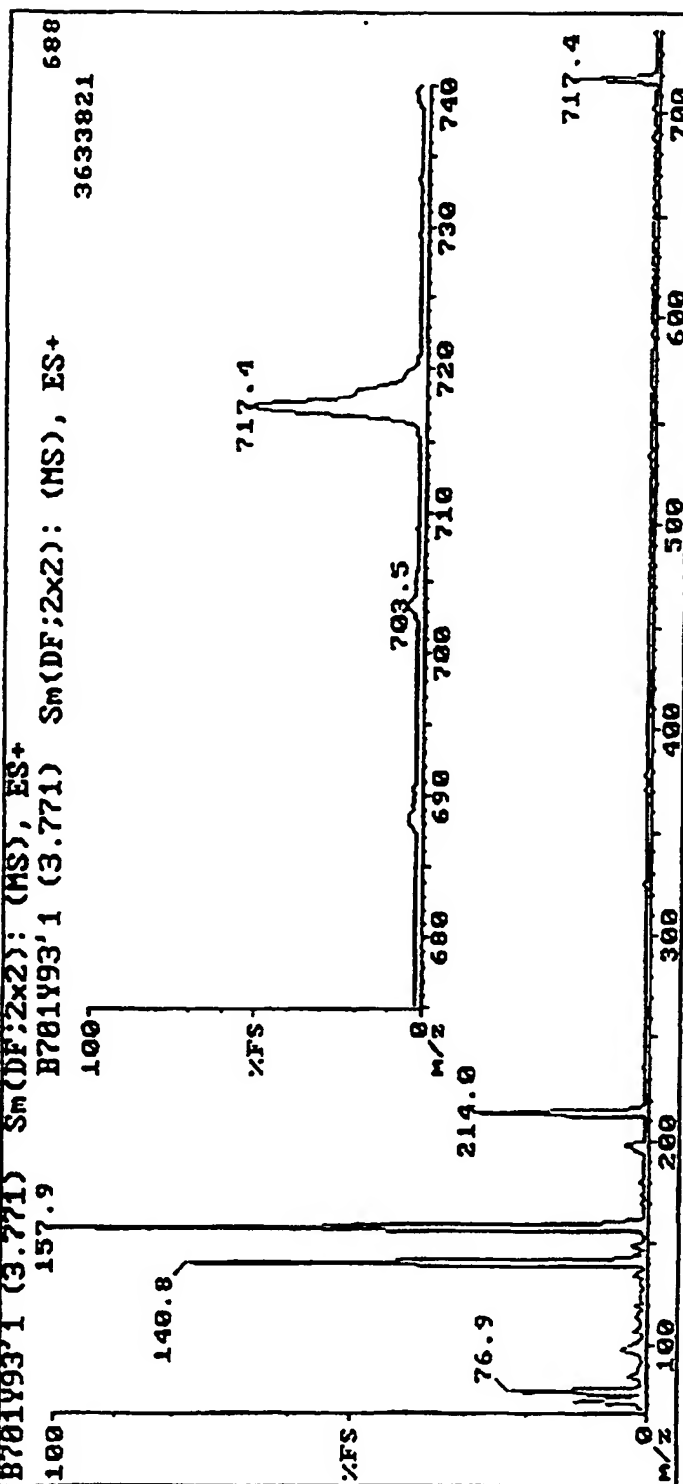
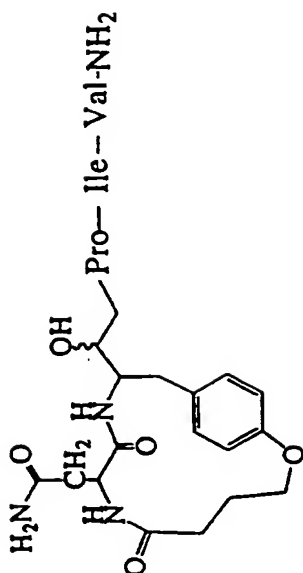


FIG. 7

8/12



18/11/1993 12:47

File: B700Y93

S93002C S-ISOMER B1=69 B2=150 R1/R2=14/14

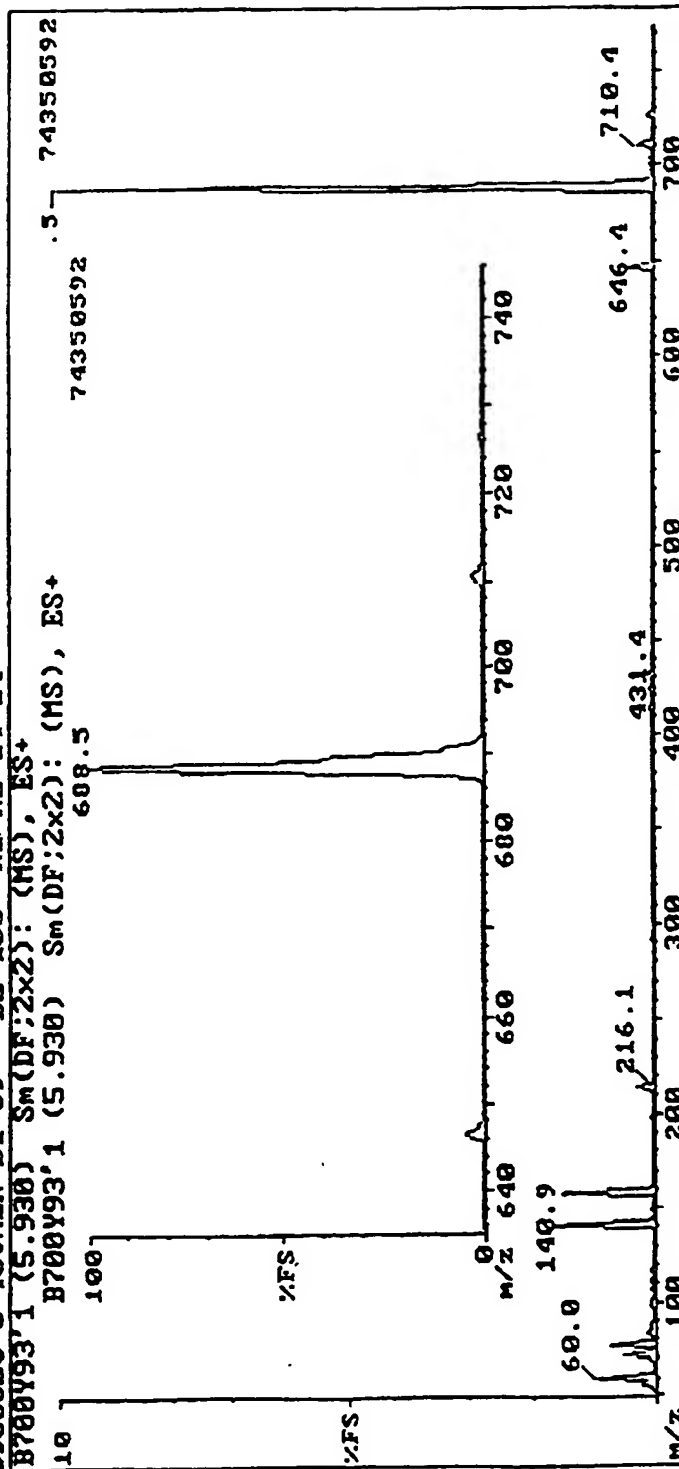
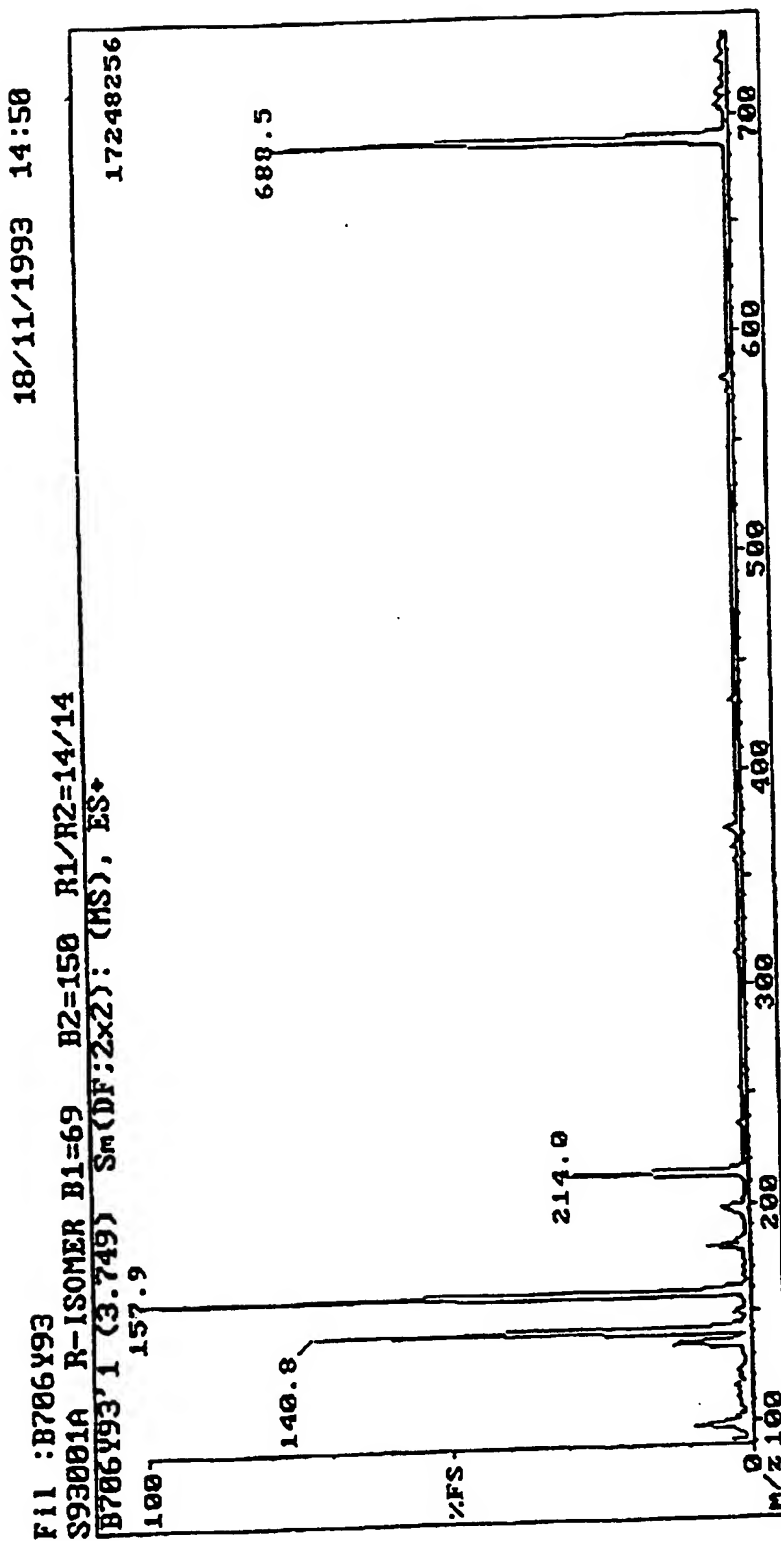
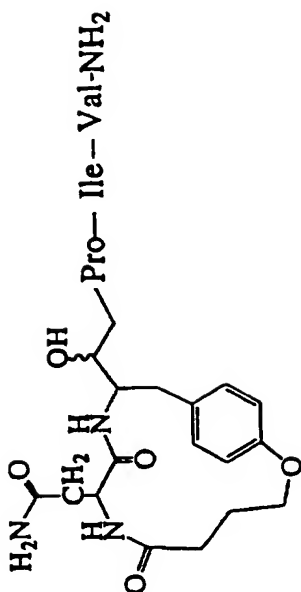


FIG. 8

9/12



516

10/12

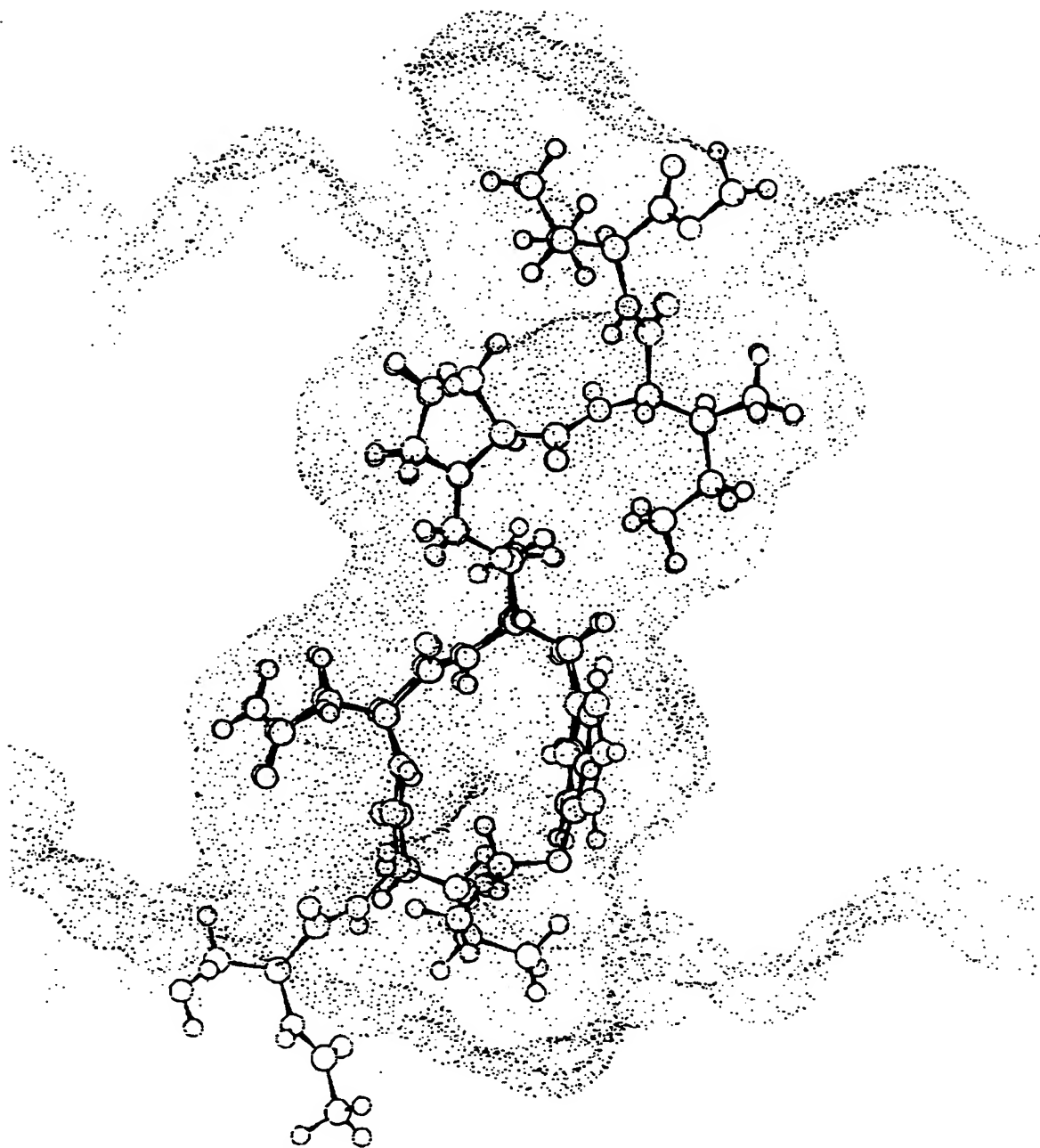


FIG. 10

11/12

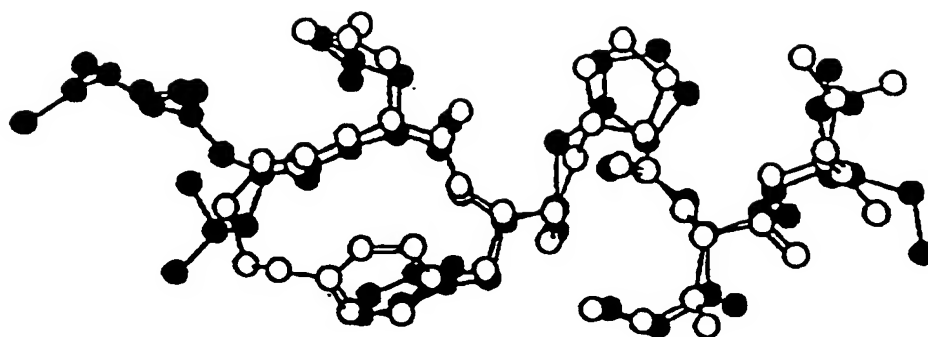


FIG. 11

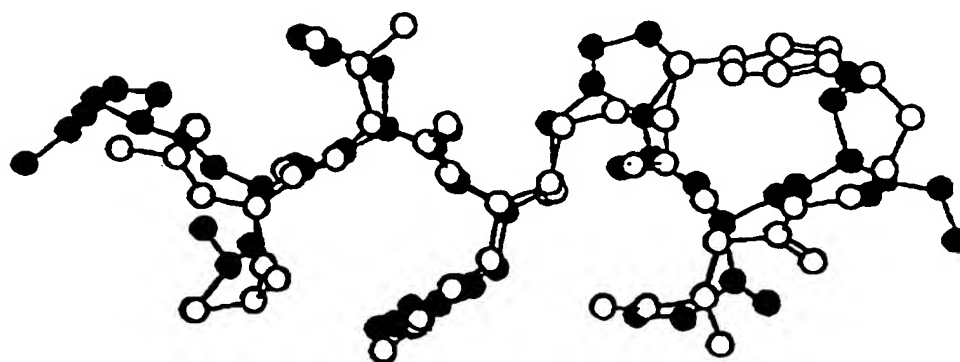


FIG. 12

12/12

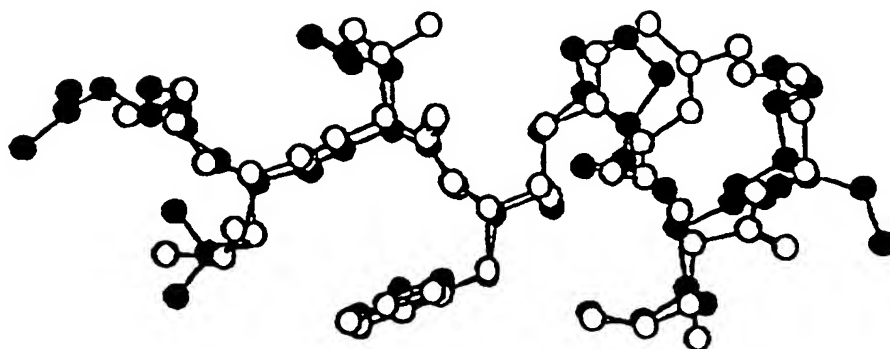


FIG. 13

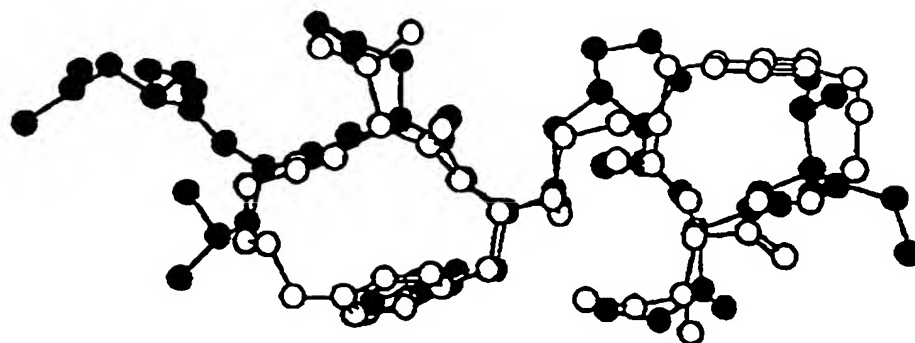


FIG. 14

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 95/00817

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl⁶: C07D 273/02; 413/06; 413/14; 513/08; 487/08
C07K 5/023; 5/078; 5/097; 7/06; 5/06

According to International Patent Classification (IPC) r to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. Cl.⁵: C07D 273/02; 413/06; 413/14; 513/08; 487/08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU : IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS-ON-LINE Sub-structure search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Proc. Natl. Acad. Sci, Vol. 87, pp. 8805-8809, November 1990. Swain et al.	5
X,P	J. Am. Chem. Soc., Vol. 117, pp. 10220-10226, October 1995. Abbenante et al.	1-5



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search

8 February 1996

Date of mailing of the international search report

15 FEB 1996

Name and mailing address of the ISA/AU
AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION
PO BOX 200
WODEN ACT 2606
AUSTRALIA Facsimile No.: (06) 285 3929

Authorized officer


MADHU K. JOGIA

Telephone No.: (06) 283 2512

